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AUTHORITY

Phylis M. Rhinehart, U.S. Army Medical Research and Materiel Command, MCMR-RMI-S[70-1Y] DCS Info. Mgmt. Ft. Detrick, MD.

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PRINCIPAL INVESTIGATOR: John A. Kepler, Ph.D.

CONTRACTING ORGANIZATION: Research Triangle Institute

Research Triangle Park, NC 27709-2194

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13. ABSTRACT *(Maximum 200*

This report summarized work carried out on contract DAMD17-93-C-3001 during the period November 15, 1995 to November 14, 1996. The purpose of the work on this contract is to prepare and fully characterize radiolabeled samples of compounds which are of current interest to the U.S. Army Medical Research and Materiel Command (USAMRMC) and to provide these compounds along with some commercially prepared compounds to investigators designated by the USAMRMC. The syntheses of carbon-14 labeled nitrogen mustard (WR-1439), [^{14}C]halofantrine (WR-171669), [^{14}C]desbutylhalofantrine (WR-178460), [^{14}C]artemether (WR-254986), and [^{14}C]artelinic acid (WR-255663) were completed during this report period. The synthesis of high specific activity tritium labeled artemisinin, and an alternate synthesis of carbon-14 labeled artemisinin were investigated. Resyntheses of [^{14}C]mefloquin (WR-142490) and [^{3}H]pyridostigmine bromide (WR-250710) were initiated.

A total of nine shipments were made, three of which required purification prior to shipment.

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FOREWORD

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Adam A. Kegler 12/10/96

PI - Signature Date

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1.0 Introduction

This report summarized work carried out on contract DAMD17-93-C-3001 during the period November 15, 1995 to November 14, 1996. The purpose of the work on this contract is to prepare and fully characterize radiolabeled samples of compounds which are of current interest to the U.S. Army Medical Research and Materiel Command (USAMRMC) and to provide these compounds along with some commercially prepared compounds to investigators designated by the USAMRMC.

The procedure followed for preparing the compounds involved first designing a synthetic scheme and then optimizing individual reactions in the synthetic scheme using nonlabeled chemicals. When all of the reactions had been optimized, a tracer run was done where a small amount of the radiolabeled starting material was diluted with non-labeled starting material, and the reaction sequence performed from beginning to end on the exact scale that was planned for the master run. Any problems which were discovered in the tracer run were worked out, and then the tracer run was repeated, if necessary, or the master run was done.

The final products were analyzed for chemical and radiochemical purity, and specific activity. Procedures used for the analyses included TLC-RAM, HPLC-RAM, UV, NMR and mass spectrometry where required. In addition to analyzing the compounds when they were first prepared, they were also analyzed prior to shipment to approved investigators.

The labeled compounds were stored at the Research Triangle Institute and sent to investigators upon request of the Project Monitor. An up-to-date list of compounds in inventory was provided to the Project Monitor each month.

The following conventions are used in this report in order to avoid confusion between nonlabeled and labeled compounds: (a) unless otherwise designated, a compound and the number associated with it represents a nonlabeled entity. (b) Numbers

and names, including partial names of labeled compounds, will be preceded by an appropriate modifier in brackets, i.e. [^{14}C]-**10** or aldehyde [^{14}C]-**10**, etc. Specifiers will be included when required for clarity, i.e. [1,2-^{3}H]-**10,** [2-^{3}H]-**10,** etc.

The terms HPLC-RAM and TLC-RAM are used when radioactivity monitors are used as detectors with otherwise conventional HPLC or TLC analyses.

2.0 Synthesis of Labeled Compounds

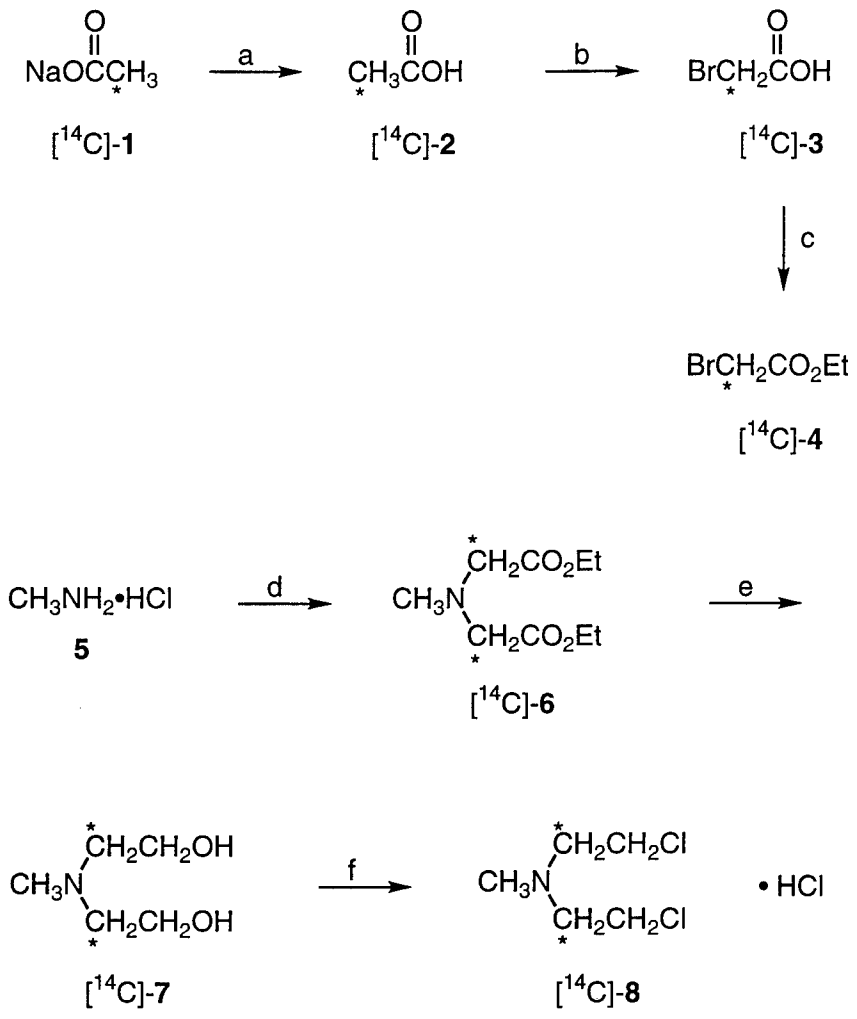
2.1 WR-1439: 2,2'-Dichloro-N-methyldi([1-^{14}C]ethyl)amine Hydrochloride; [chloroethyl-1-^{14}C]Nitrogen Mustard; ([^{14}C]-8)

In our last report[1] we described the master synthesis of [chloroethyl-1-^{14}C]nitrogen mustard ([^{14}C]-**8**, Chart 1) up to the preparation of diol [^{14}C]-**7**. We also stated that since we expected the diol to be more stable than [^{14}C]-**8**, that only a portion of it would be converted to the final product with the remainder being reserved for use at a later date. Treatment of a 28 mCi sample of [^{14}C]-**7** with thionyl chloride afforded crude [^{14}C]-**8** after workup. The crude salt was purified by multiple crystallizations and dilutions with nonlabeled **8** to yield 58 mg (5.1 mCi, 18% radiochemical yield) of pure [^{14}C]-**8** with specific activity of 88.5 μCi/mg and of 97% radiochemical purity. This material was entered into inventory as lot no. CT-8136-181-3. A second crop (~ 6 mCi, 32% radiochemical yield) of 95% radiochemical purity was also obtained. Further details can be found in the accompanying synthesis report in the Appendix.

2.2 WR-171669: 1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N,N-di-n-butyl-amino)[1-^{14}C]propyl]phenanthrene Hydrochloride; [^{14}C]Halofantrine, ([^{14}C]-16)

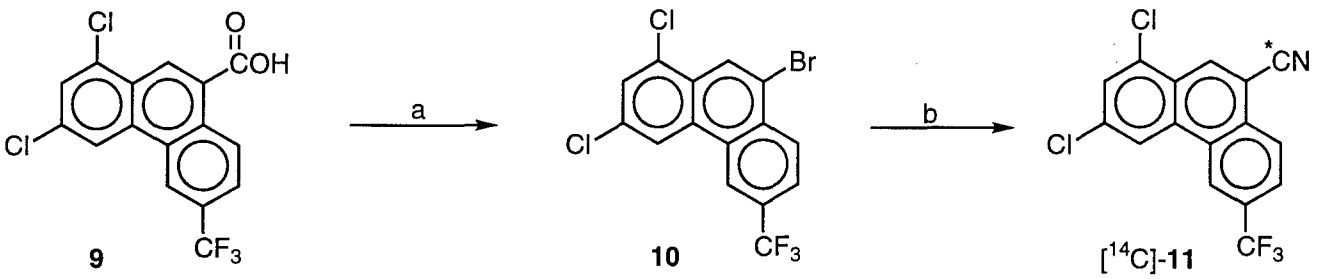
In our last report[1] we described the master synthesis of [^{14}C]halofantrine ([^{14}C]-**16**, Chart 2) up to the preparation of alcohol [^{14}C]-**12**. Oxidation of [^{14}C]-**12** with cerium ammonium nitrate afforded aldehyde [^{14}C]-**13** in 71% yield after crystallization. Analysis of this material by TLC-RAM indicated that further purification was required. It was combined with a second crop of [^{14}C]-**13** and chromatographed. Fractions containing pure [^{14}C]-**13** were combined to afford material of >98% radiochemical purity. Fractions of lesser purity were combined to give material that was 91% radiochemically pure by TLC-RAM. This material was further purified by crystallization and combined with the

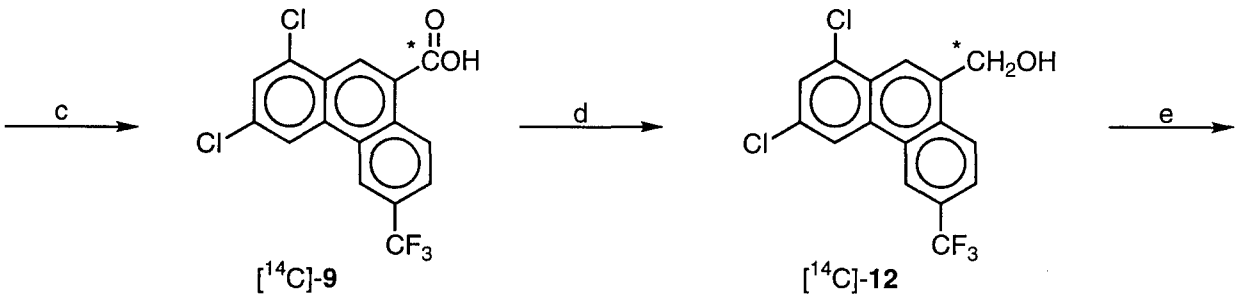
Chart 1

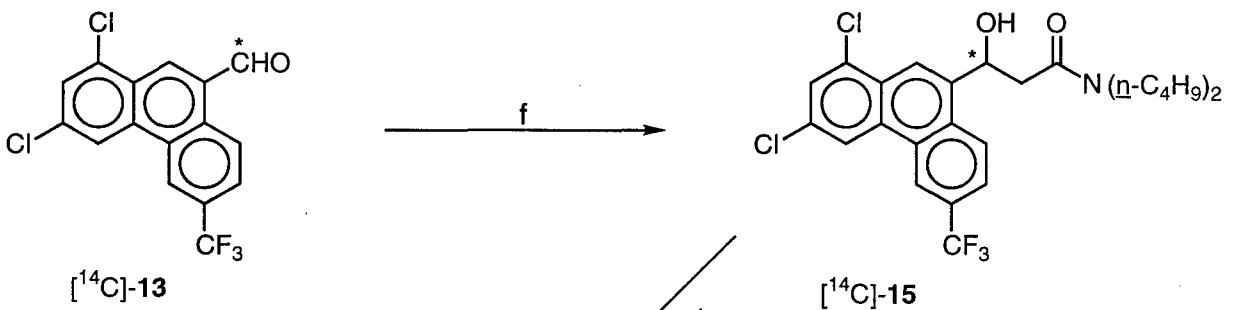


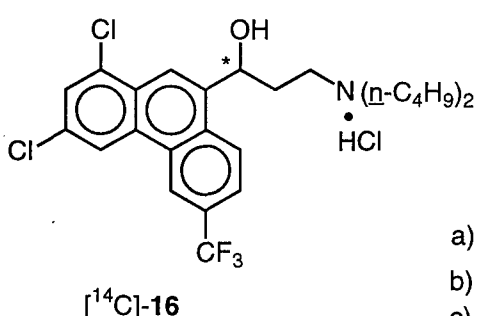
a) H_3PO_4, P_2O_5
b) $(CF_3CO)_2O$, Br_2
c) EtN_2
d) [^{14}C]-**4**, Et_3N
e) $LiAlH_4$
f) $SOCl_2$

Chart 2









a) Br_2, HgO, hυ
b) $(Ph_3P)_4Pd$, KCN, 18-crown-6, Al_2O_3
c) H_3O^+
d) BH_3•THF
e) $(NH_4)_2Ce(NO_3)_6$, (CAN)
f) $BrZnCH_2\overset{O}{\overset{\|}{C}}N(\underline{n}\text{-}C_4H_9)_2$ (**14**)
g) BH_3•THF
h) HCl

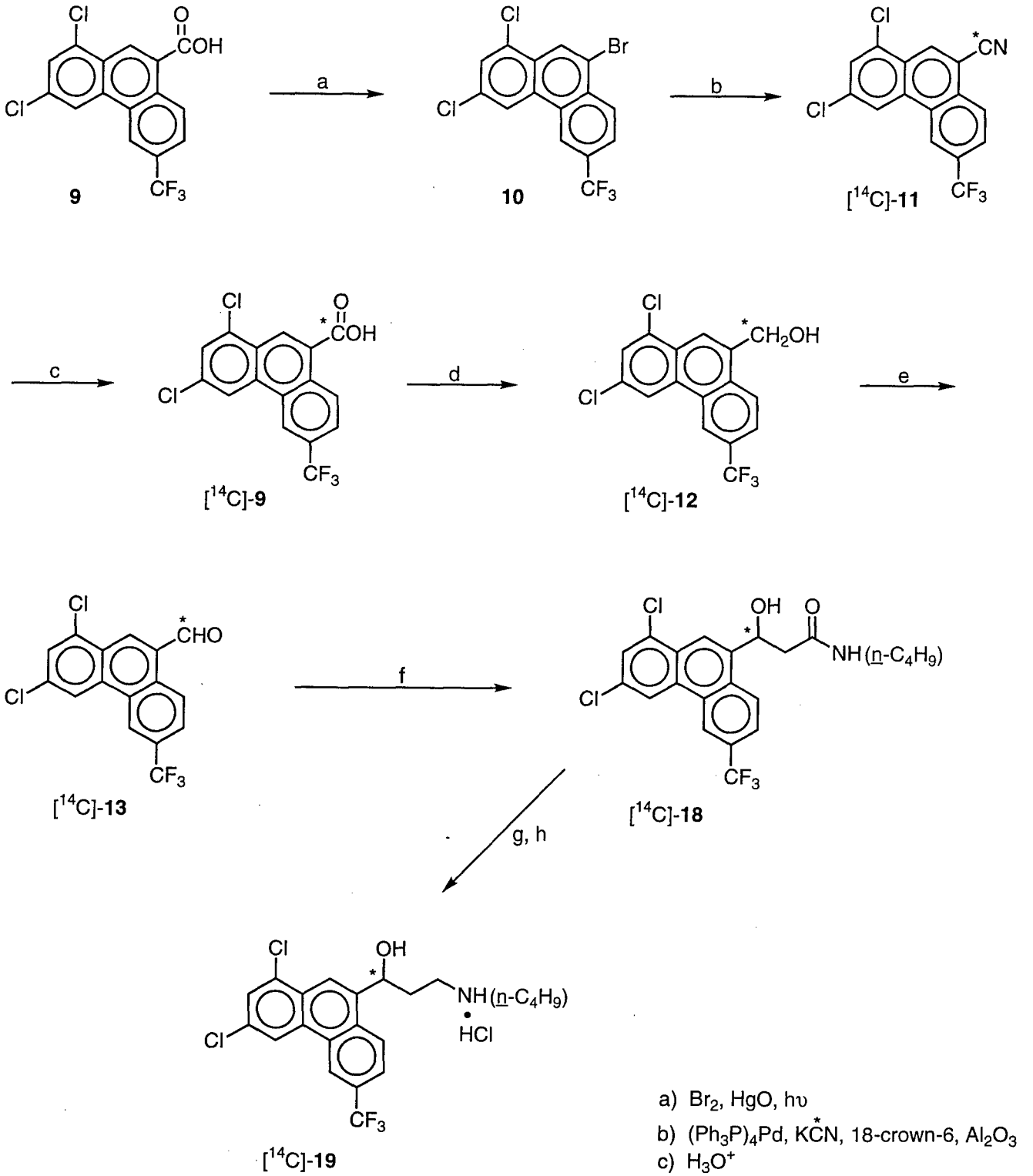
pure [^{14}C]-**13** from the chromatography to afford a 73% radiochemical yield of [^{14}C]-**13** that was 97% radiochemically pure. Reaction of [^{14}C]-**13** with the Reformatsky reagent **14** gave amide [^{14}C]-**15** in 88% radiochemical yield and 99% radiochemical purity after chromatography. Reduction of [^{14}C]-**15** with borane-THF complex afforded a 72% radiochemical yield of [^{14}C]halofantrine after workup and crystallization of the hydrochloride salt. This material was diluted with nonlabeled halofantrine to afford 351 mg (19.5 mCi) of [^{14}C]halofantrine (lot no. CF-8144-182-3) with specific activity of 29.8 mCi/mmol, 55.5 μCi/mg and 98% and 97% radiochemically pure by HPLC-RAM and TLC-RAM, respectively. The ^{1}H NMR spectrum of the product was identical with that of an authentic sample of halofantrine. Further details can be found in the accompanying synthesis report in the Appendix.

2.3 WR-178460: 1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N-n-butylamino)[1-^{14}C]propylphenanthrene Hydrochloride; ([^{14}C]-19)

The synthesis of carbon -14 labeled WR-178460 ([^{14}C]-**19**) was accomplished by the method outlined in Chart 3. The reaction scheme is essentially the same as that used for preparing [^{14}C]halofantrine. Since aldehyde [^{14}C]-**13** is a common intermediate, the development work focused on the last two steps of the reaction scheme.

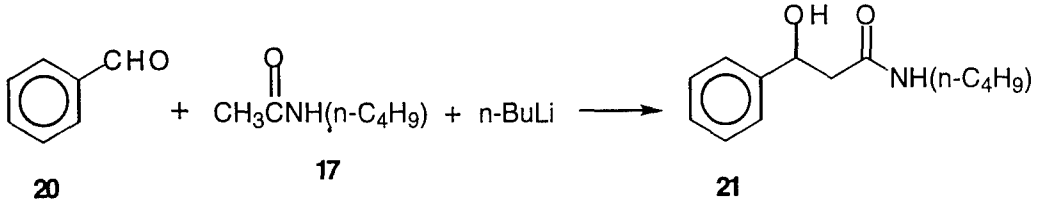
The results of several runs of the reaction of aldehyde **13** with the dilithium salt of N-n-butylacetamide (**17**) indicated that the best yield was obtained by carrying out the reaction under strictly anhydrous conditions at -5 °C and working it up promptly after completion. Allowing the reaction mixture to stir overnight at room temperature appeared to cause decomposition and much reduced yields. Thus, N-n-butylacetamide (**17**) was treated with two molar equivalents of n-butyllithium and allowed to stir for 2 h at -5 °C under an argon atmosphere. A solution of aldehyde **13** in freshly distilled THF was added dropwise to the resulting suspension and stirring at -5 °C was continued for an additional 2 h. The solution was allowed to warm to room temperature (30 min) and

Chart 3



a) Br_2, HgO, hυ
b) $(Ph_3P)_4Pd$, K*CN, 18-crown-6, Al_2O_3
c) H_3O^+
d) BH_3•THF
e) $(NH_4)_2Ce(NO_3)_6$, (CAN)
f) n-BuLi, $CH_3\overset{O}{\overset{\|}{C}}NH(n\text{-}C_4H_9)$ (**17**)
g) BH_3•THF
h) HCl

was quenched with 15% ammonium acetate solution. Extraction and chromatography of the crude product afforded a 72% yield of pure **18**. Unfortunately, this high yield of **18** was not reproducible. Consequently, further work was focused on this reaction. Because of the limited supply of aldehyde **13**, the alkylation reaction was explored by using benzaldehyde (**20**) as a model compound. Reaction of **20** (3.1 mmol) with 1.6 molar equivalents of **17** and 3.2 molar equivalents of n-butyllithium at -5 °C under



20 **17** **21**

strictly anhydrous conditions gave 57% and 55% yields of **21** after workup and purification by chromatography (entries 1 and 2, Table 1). When this reaction was repeated with a 3.5 molar equivalents of **17** and 6.0 molar equivalents of n-butyllithium 52% and 61% yields of **21** were realized (entries 3 and 6, Table 1). The yield of **21** was significantly reduced as estimated by TLC when the alkylation reaction was carried out at -20 °C or at 0 °C (entries 4 and 5, Table 1). These results suggest that the alkylation reaction is not particularly sensitive to the amount of excess **17** or n-butyllithium, but is sensitive to temperature. Although the 50-60% yield of product is lower than desired, it was at least consistent under the conditions described above, and so we considered it to be acceptable for the radiosynthesis. Thus, aldehyde **13** (1.5 mmol) was alkylated with 3.5 molar equivalents of **17** and 6.0 molar equivalents of n-butyllithium. Workup and chromatographic purification of the product however, afforded only a 27% yield of **18**. The major side product from this reaction was the alcohol **12**, which presumably arises from a Cannizzaro reaction of aldehyde **13**. We confirmed that a Cannizzaro reaction of **13** is taking place by isolation of the expected companion product, carboxylic acid **9**, from the reaction mixture . The Cannizzaro reaction normally takes place by

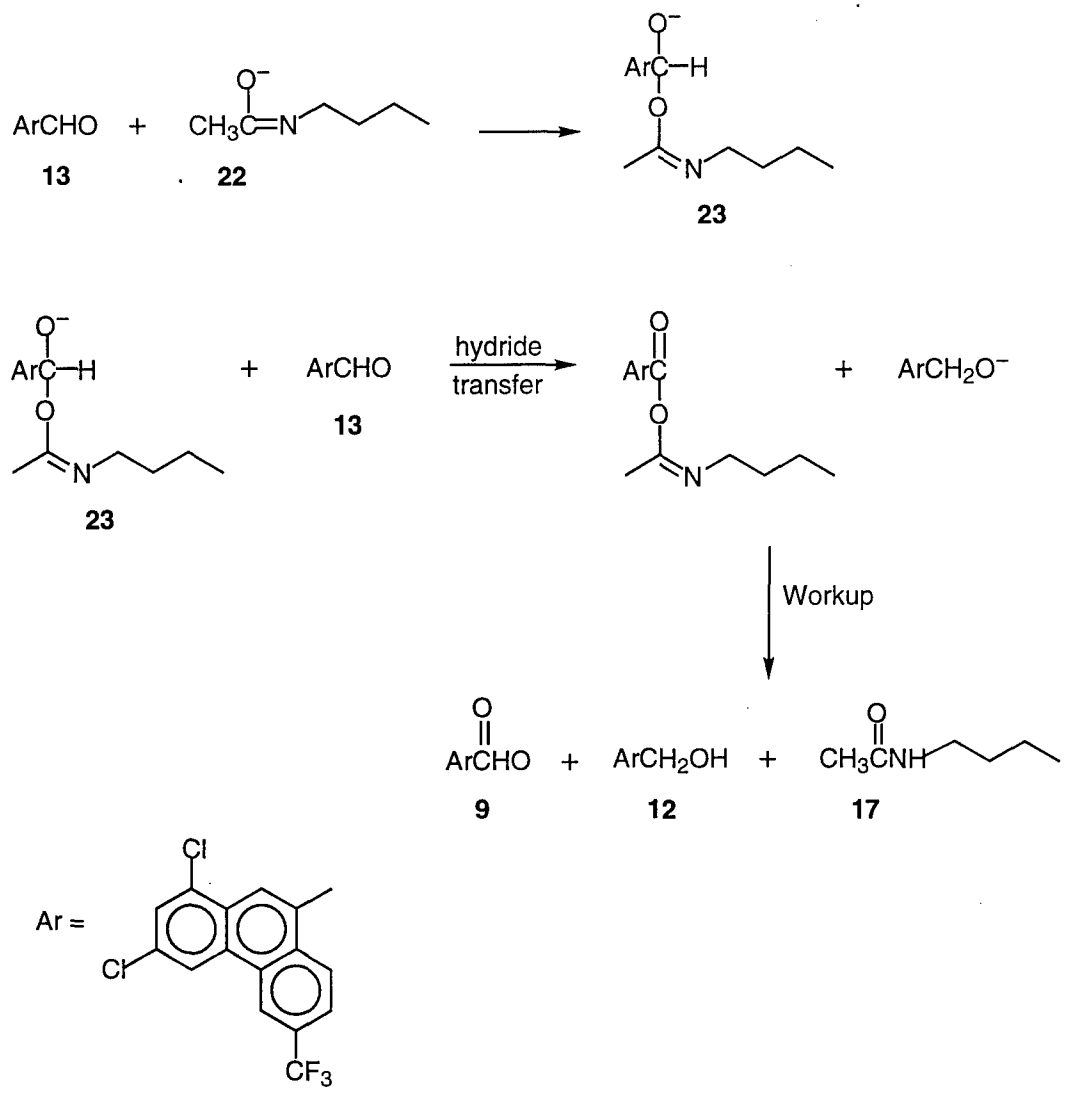
Table 1

Entry	Amide 17	BuLi	Benzaldehyde	Temp.	Yield
1	5.00 mmol (1.6)[a]	10.0 mmol (3.2)	3.13 mmol (1)	-5 °C	57%
2	5.2 mmol (1.6)	10.0 mmol (3.2)	3.13 mmol (1)	-5 °C	55%
3	11.0 mmol (3.5)	18.8 mmol (6)	3.13 mmol (1)	-5 °C	51.8%
4	11.0 mmol (3.5)	16.20 mmol (5.2)	3.13 mmol (1)	-20 °C	Not worked up, estimated yield < 30%
5	11.0 mmol (3.5)	16.24 mmol (5.2)	3.13 mmol (1)	0 °C	Not worked up, estimated yield < 30%
6	11.0 mmol (3.5)	19.0 mmol (6.1)	3.13 mmol (1)	-5 °C	60.7%

a)The numbers in parentheses are the molar ratio of the reagents.

reaction of a non-enolizable aldehyde with hydroxide or alkoxide ion.[2] Scrupulous precautions were taken to keep the reaction anhydrous, and so the source of the base for the Cannizzaro reaction was puzzling. The alkylation of **13** is carried out with an excess of **17** with respect to n-butyllithium and to **13**. Because of this, there is some of the monoanion of **17**, i. e. **22**, present in the reaction mixture. We speculated that **22** could catalyze the disproportionation of **13** by the mechanism shown in Chart 4. This mechanism parallels that proposed for the hydroxide and alkoxide induced Cannizaro reaction.[2] To test this hypothesis, a sample of **13** was treated with **22** generated from an equivalent of n-butyllithium and **17**. Analysis of this reaction after workup by TLC and NMR indicated the formation of alcohol **12** and acid **9**. These results support the hypothesis, but are not definitive. Based on these results, alkylation of **13** was carried out using a two-fold excess of **17** and four equivalents of n-butyllithium to insure that no monoanion **22** was present. Under these conditions the yield of **18** was improved from

Chart 4



27% to 42%. Reduction of **18** with diborane followed by an acidic workup and crystallization of the product afforded a 74% yield of the target compound **19**.

Based on the above results a tracer synthesis of [^{14}C]-**19** was started. Ceric ammonium nitrate oxidation of alcohol [^{14}C]-**12** afforded a 52% radiochemical yield of aldehyde [^{14}C]-**13** after purification by chromatography. Reaction of [^{14}C]-**13** with a two fold excess of **17** and four equivalents of n-butyllithium gave a 72% radiochemical yield of [^{14}C]-**18**. This increase in the yield of **18** may be due to the addition of aldehyde

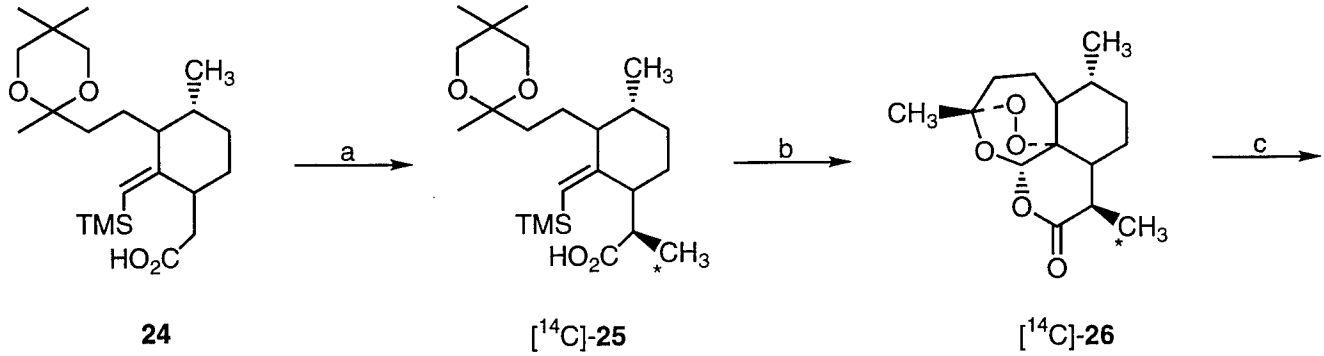
[^{14}C]-**13** rather rapidly by a cannula compared to the dropwise addition used in the pilot studies.

Reduction of amide [^{14}C]-**18** with borane-THF complex gave an 83% radiochemical yield of [^{14}C]-**19** after several recrystallizations from ethanol. In the master synthesis, alcohol [^{14}C]-**12** was oxidized with ceric ammonium nitrate (CAN) to afford aldehyde [^{14}C]-**13** in 70% radiochemical yield after purification by flash chromatography. Reaction of [^{14}C]-**13** with the dilithium salt of N-butylacetamide (**17**) using the procedures developed in the tracer run gave amide [^{14}C]-**18** in 61% radiochemical yield after chromatography. Borane reduction of [^{14}C]-**18** gave a 3:1 mixture of [^{14}C]-**19** and unreacted [^{14}C]-**18**. The [^{14}C]-**18** was separated from the product by extraction with ethyl acetate. The recovered [^{14}C]-**18** was diluted with nonlabeled **18** and subjected to the borane reduction. The [^{14}C]-**19** isolated from this reduction was combined with the product from the first reduction and purified to afford a 79% radiochemical yield (16 mCi) of carbon-14 labeled WR-178460 (lot no. CF-8448-129-4). The product is 96.5% radiochemically pure by TLC-RAM and 98.2% radiochemically pure by HPLC-RAM. The specific activity is 36.4 mCi/mmol (75.8 μCi/mg). Experimental details may be found in the accompanying synthesis report in the Appendix.

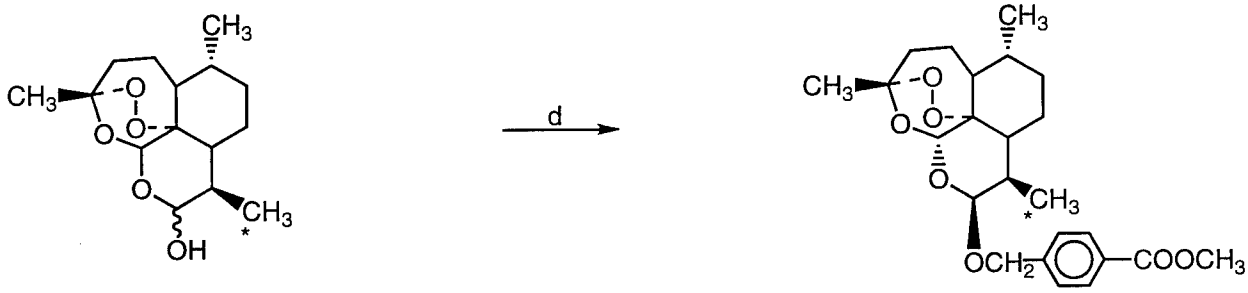
2.4 WR-255663: [16-^{14}C]Artelinic Acid; ([^{14}C]-29)

We also completed the synthesis of carbon-14 labeled artelinic acid (Chart 5, [^{14}C]-**29**). [16-^{14}C]Artemisinin ([^{14}C]-**26**) (2.4 mCi) was reduced with sodium borohydride to give 1.91 mCi (80% yield) of [^{14}C]dihydroartemisinin ([^{14}C]-**27**) after chromatography. Treatment of [^{14}C]-**27** with methyl 4-(hydroxymethyl)benzoate gave methyl [^{14}C]artelinate ([^{14}C]-**28**) in 96% radiochemical yield after chromatography. The [^{14}C]-**28** was hydrolyzed with potassium hydroxide in aqueous methanol to obtain [^{14}C]-**29**. Purification of this material by preparative HPLC gave pure [^{14}C]-**29** in 86% radiochemical yield. This material was diluted with nonlabeled **29** and recrystallized from ethyl acetate-hexane to obtain 45 mg of [^{14}C]-**29** as white crystals with specific

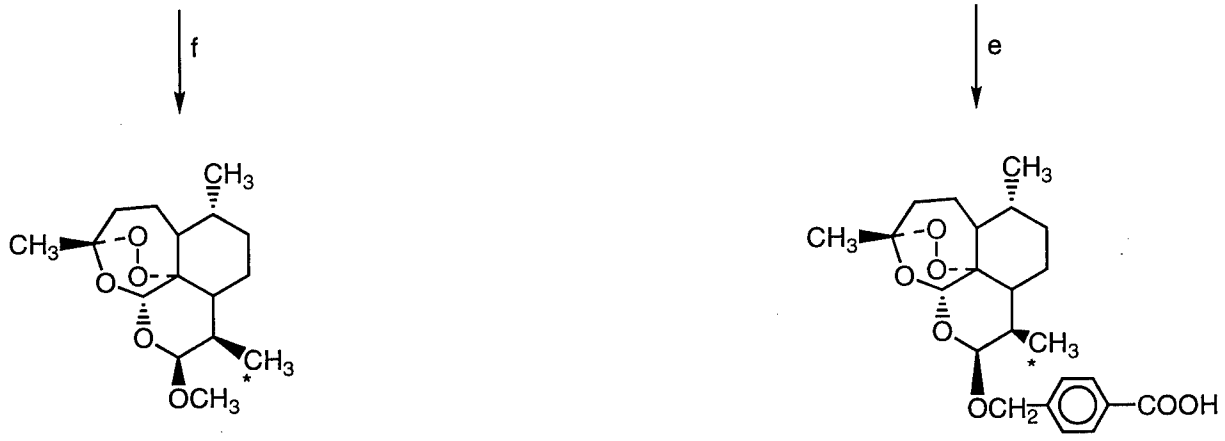
Chart 5



24 [^{14}C]-**25** [^{14}C]-**26**



[^{14}C]-**27** [^{14}C]-**28**



[^{14}C]-**30** [^{14}C]-**29**

a) LDA, $^{*}CH_3I$

b) O_2/O_3, H_3O^+

c) $NaBH_4$, MeOH

d) $BF_3 \bullet Et_2O$, $HOCH_2$-C$_6$H$_4$-$COOCH_3$

e) 5% KOH/CH_3OH

f) $BF_3 \bullet Et_2O$, MeOH

activity of 32 μCi/mg and radiochemical purity > 98% by HPLC. This material was entered into the inventory as CT-8440-43. Further details can be found in the accompanying synthesis report in the Appendix.

2.5 WR-254986: [16-^{14}C]Artemether; ([^{14}C]-30)

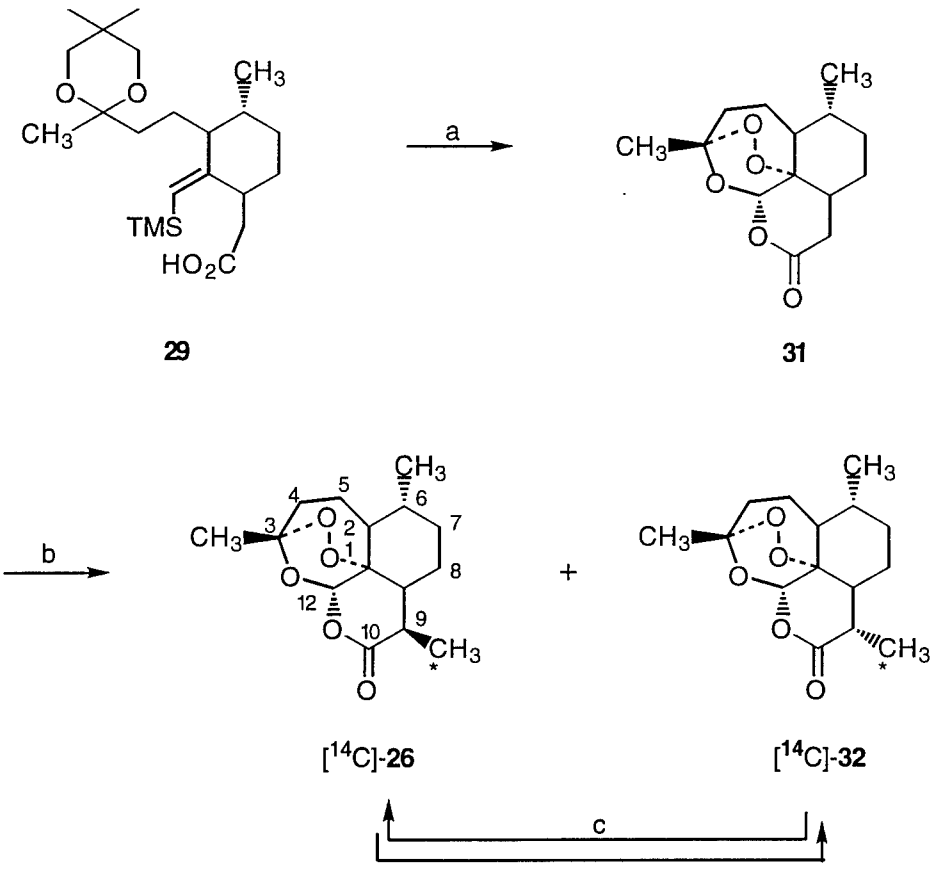
The synthesis of carbon-14 labeled artemether (Chart 5, [^{14}C]-**30**) was accomplished this report period. [^{14}C]Artemisinin ([^{14}C]-**26**, 1.0 mCi) was reduced with sodium borohydride to give 835 μCi (83.5% yield) of [^{14}C]dihydroartemisinin ([^{14}C]-**27**) after chromatography. Treatment of ([^{14}C]-**27**) with methanol in the presence of boron trifluoride etherate gave [^{14}C]-**30** in 99% crude radiochemical yield.

This material was purified by flash chromatography to give pure β-isomer of [^{14}C]-**30** in 54% radiochemical yield. The overall radiochemical yield from [16-^{14}C]-artemisinin was 45%. A total of 451 μCi of material with specific activity of 96 μCi/mg was entered into the inventory as lot no. CT-8440-77-1. The radiochemical purity was 98% by HPLC-RAM. Further details can be found in the synthesis report in the Appendix.

2.6 WR-249309: [16-^{14}C]Artemisinin; ([^{14}C]-26)

Investigation of an alternate method of preparing [16-^{14}C] artemisinin has been carried out. The scheme (Chart 6) was originally investigated for the synthesis of high specific activity tritium labeled artemisinin but was abandoned because of the difficulty encountered in carrying out the microscale alkylation reaction.[1] It is now only being considered for preparing carbon-14 labeled artemisinin, because it requires fewer steps with radioactive material and has the potential of giving better radiochemical yields than the method that we are presently using (vide supra). Epimerization of the crude alkylated product mixture obtained by reacting desmethylartemisinin (**31**, 100 mg, 0.37 mmol) with two equivalents of methyl iodide afforded a 25% isolated yield of artemisinin (**26**) and a 44% yield of epiartemisinin (**32**). No further epimerization was

Chart 6



29 **31**

[^{14}C]-**26** [^{14}C]-**32**

a) O_3
b) LDA, *CH_3I
c) LDA

carried out on the recovered epiartemisinin. In this case alkylation of **31** with methyl iodide was not done on the vacuum line. We next investigated adapting this procedure to the restrictions imposed by using radioactive materials and vacuum line techniques. A major concern was dealing with the excess [^{14}C]methyl iodide in a safe manner. We found that the excess methyl iodide could be distilled from the reaction mixture at -50 to -55 °C at 0.01 Torr on the vacuum line. In a test reaction, **31** (269 mg, 1 mmol) was reacted with 1.22 equivalent of LDA at -78 °C and the resulting anion was treated with two equivalents of methyl iodide on the vacuum line at -78 °C for 2 h and then at -62 °C for 1 h. Unreacted methyl iodide was vacuum transferred at -53 to -50 °C into a flask

containing 1-methylpiperidine over 50 min. The reaction mixture was treated with 0.1 N hydrochloric acid and extracted into methylene chloride. Evaporation of the methylene chloride afforded 230 mg of a yellow-brown solid. HPLC analysis of this product showed that it contained **31**, **26** and **32** in a ratio of 7:1:92, respectively. The HPLC profile was quite similar to that of the product from the smaller scale reaction of **31** with two equivalents of methyl iodide done without the vacuum line. In the later case **31**, **26** and **32** were obtained in a ratio of 5:2:92, respectively, and the yield of the crude product was 97 mg. The 230 mg crude product from the vacuum line reaction was equilibrated with 1.22 mmol of LDA at -78 °C for 2.5 h. HPLC analysis of the mixture (208 mg) obtained from this epimerization reaction showed little epimerization had occurred. The ratio of **31, 26** and **32** was 1:3:17, respectively whereas the corresponding ratio from the smaller scale epimerization reaction was 1:13:19, respectively. The crude 208 mg epimerization mixture was subjected to another equilibration with LDA at -60 to -51 °C for 2.5 h. HPLC analysis of the crude mixture from this reaction showed the ratio of **31**, **26** and **32** to be 1:5:14, respectively indicating that more artemisinin had formed. Purification of this mixture (182 mg) afforded pure **26** (44 mg, 15.5% yield) and **32** (94.3% pure, 103 mg, 36.4% yield). The combined yield of **26** and **32** from **31** was 51.9%, whereas the corresponding yield from the non vacuum line smaller scale synthesis was 67% (**26**, 25% yield and **32**, 42% yield). Next the sample of 94.3% pure epiartemisinin was treated with 1.22 equivalents of LDA at -70 to -55 °C over 2.5 h. Almost no epimerization of **32** was observed this time.

Since the epimerization of epiartemisinin to artemisinin was not reliable, further study of this reaction was undertaken. Epiartemisinin was prepared in 45% yield after purification by treatment of **26** with 20% excess of LDA. The isolated **32** was 94% pure. Samples of **32** (50 mg, 0.18 mmol) were treated with various amounts of LDA at -78 °C. The crude mixtures were analyzed by HPLC before isolation of **32** and **26** by chromatography. The results are given in Table 2.

Table 2

Epimerization of Epiartemisinin (32) to Artemisinin (26)

Run	LDA (equiv.)	Ratio 32:26 (HPLC)	Crude Yield (mg)	Time (h)	Milligrams Isolated (% Yield, % Purity) 32	26	Total Yield (%)
1[a]	0.5	240:1	58	2.5	-----	-----	-----
2	2.4	1.6:1	32	2.5	14 (28, 95)	8.6 (17, 92)	45
3	4.8	1.2:1	26	2.5	9.3 (19, 90)	9.1 (18, 88)	37
4	4.8	1.2:1	33	1	10 (20, 93)	9.0 (18, 88)	38

a) Reaction done at -78 °C to -50 °C

The results indicate the following: 1) 0.5 equivalents of LDA is not sufficient to cause epimerization (Run 1); 2) more epimerization occurs at higher LDA concentration (compare Runs 2 and 3); and 3) at higher LDA concentration (Runs 3 and 4) there is no appreciable difference between 1 h and 2.5 h reaction times. The total recovery of **32** and **26** from the epimerization of **32** is low when compared to the epimerization of **26** where 73% recovery of material is realized.

Further work concentrated on developing conditions that would provide a better yield of artemisinin along with good recovery of epiartemisinin. A second set of experiments was carried out similar to the first set. The results are summarized in Table 3. In these experiments, samples of epiartemisinin (50 mg, 0.18 mmol) were treated with various amounts of LDA at -78 °C in a constant volume of dry THF for different time periods (see Table 3). At the end of the specified time, the reaction mixture was treated with 0.1 N hydrochloric acid , evaporated to dryness and analyzed by HPLC. The crude product mixtures were purified by column chromatography. In the case of Run 5, the reaction mixture was stirred with a solution of 2,6-di-*tert*-butylphenol (0.25 mmol) in dry THF (0.2 ml) at -78 °C for 10 min before the regular workup. In the case of Run 6, the reaction mixture was stirred with a solution of 2,6-diadamantyl-4-methylphenol

(0.25 mmol) in dry THF (1.6 ml) at -78 °C for 10 min before the regular workup. These two runs were done to determine if quenching the epimerization mixture with a sterically hindered acid would have an effect on the ratio of artemisinin to epiartemisinin.

The results indicate the following: 1) shorter reaction times with LDA give somewhat improved yields of artemisinin and recovered epiartemisinin (compare Runs 1 and 2, Table 3); 2) increasing the amount of LDA gives a better ratio in favor of artemisinin but a somewhat lower total yield (compare Runs 3 and 4, Table 3); 3) quenching the reaction mixture with 2,4-di-*tert*-butylphenol prior to workup affords better total recovery (compare Runs 2 and 5, Table 3), and even though the epiartemisin:artemisinin ratio is less favorable than in Run 2 (Table 3), the amount of artemisinin recovered in Run 5 (Table 3) is not significantly different; and 4) the more hindered acid, 2,6-diadamantyl-4-methylphenol, gave less favorable results (Run 6, Table 3).

The epimerization reaction was done on a larger scale. Thus, epiartemisinin (283 mg, 1 mmol) was treated with 1.2 mmol of LDA at -78 °C for 1 h. A solution of 2,6-di-*tert*-butylphenol (288 mg, 1.4 mmol) in dry THF (1.1 mL) was added and the mixture was stirred at -78 °C for another 1.5 h. Workup and column purification afforded artemisinin (66 mg, 23% yield, 91% pure) and epiartemisinin (170 mg, 60% yield, 92% pure).

Since using excess LDA afforded a better ratio in favor of artemisinin, (Run 4, Table 3) and 2,6 di-*tert*-butylphenol quench gave a good recovery (Run 5, Table 3) we did further experiments under these combined conditions. Epiartemisinin (50 mg, 0.18 mmol) was was treated with LDA (2.4 equiv.) at -78 °C for 15 min. The reaction was quenched with 2,6 -di-*tert*-butylphenol (0.5 mmol) and HPLC analysis of the crude product indicated a 2.5:1 ratio of epiartemisinin to artemisinin. A 1.6 :1 ratio of epi-artemisinin to artemisinin was obtained when the same reaction was done without the 2,6-di-*tert*-butylphenol quench. Purification of the reaction mixture by chromatography afforded **32** (26 mg, 52% yield) and **26** (11 mg, 22% yield). The mass recovery (74%

Table 3

Epimerization of Epiartemisinin (32) to Artemisinin (26)

Run	LDA (equiv.)	Ratio 32:36 (HPLC)	Time (h)	Milligrams Isolated (% Purity) 32	26	Total Yield (%)
1	1.3	2.1:1	2.5	23.3 (98)	12.1 (97)	71
2	1.3	2.1:1	1	25.6 (95)	13.9 (96)	79
3	1.3	2.3:1	0.25	24.6 (97)	9.9 (87)	69
4	2.4	1.6:1	0.25	18.8 (91)	13.5 (88)	65
5	1.3	3.1:1	1	30.5 (99)	13.3 (86)	88
6	1.3	3.5:1	1	28 (94)	6.8 (91)	70

yield) from this reaction was better than the 65% yield recovery from the reaction with the hydrochloric acid quench. Next the epimerization was done with LDA (4.8 equiv.) at -78 °C for 15 min followed by 2,6-di-*tert*-butylphenol quench (1 mmol). However the HPLC of the crude mixture showed considerable decomposition even though a better ratio (1.3:1) of epiartemisinin to artemisinin was observed. Further purification afforded **26** (8.5 mg, 17% yield) and **32** (8.6 mg, 17% yield).

The epimerization reaction was again done on a larger scale. Thus, epiartemisinin (283 mg, 1 mmol) was treated with 2.4 mmol of LDA at -78 °C for 15 min. A solution of 2,6-di-*tert*-butylphenol (576 mg, 2.8 mmol) in dry THF (2.2 mL) was added and the mixture was stirred at -78 °C for another 15 min. Workup and purification afforded artemisinin (81.6 mg, 29% yield, 96% pure) and epiartemisinin (100.8 mg, 36% yield, 90% pure). A similar reaction done earlier with 1.2 mmol of LDA had afforded artemisinin (66 mg, 23% yield, 91% pure) and epiartemisinin (170 mg, 60% yield, 92% pure). Even though a better yield of artemisinin was obtained from the reaction with 2.4 mmol of LDA than with 1.2 mmol of LDA, we have decided to employ the conditions of the 1.2 mmol LDA reaction for the radiolabeled synthesis due to the better mass

recovery (83% vs 65% yield) which will ultimately result in a better yield of artemisinin through recycling recovered epiartemisinin.

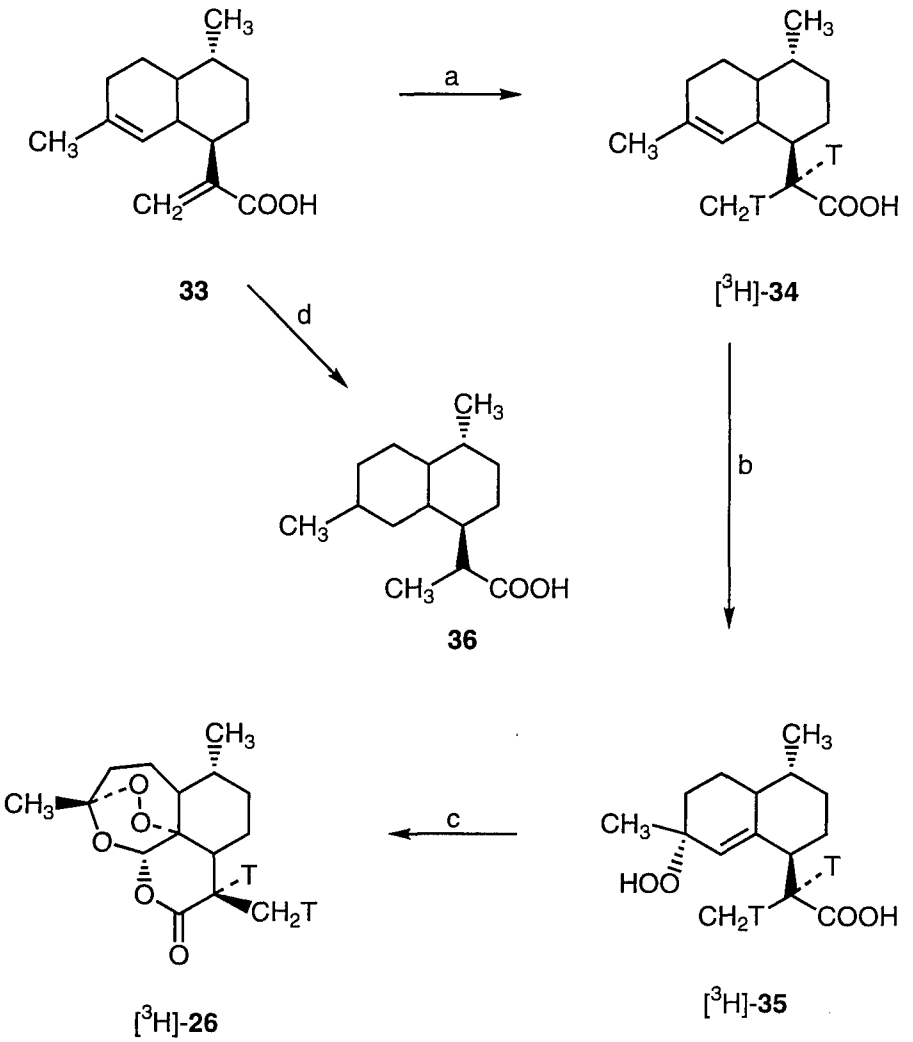
The scheme shown in Chart 6 was tested on the scale to be used for the radiosynthesis. Desmethylartemisinin (**31**, 269 mg, 1 mmol) was reacted with 1.2 mmol of LDA at -78 °C and the resulting anion was treated with 2 mmol of methyl iodide on the vacuum line at -78 °C to -70 °C for 3 h. Unreacted methyl iodide was vacuum transferred at -55 °C to -50 °C into a flask containing a THF solution of 1-methylpiperidine over 50 min. The residue was diluted with dry THF and then treated with 1 N hydrochloric acid. Evaporation of the solvent afforded a yellow solid which contained **31**, **26** and **32** in a ratio of 6:1:93, respectively. Purification by column chromatography gave **32** (227 mg, 80% yield, 89% pure) as a white solid. This material which contained 5% of desmethylartemisinin was equilibrated with 1.2 equivalents of LDA at -78 °C for 1 h. The reaction mixture was quenched with 2,6-di-*tert*-butylphenol and worked up. HPLC analysis of the crude product however, revealed that considerable decomposition had occurred. The ratio of **32** to **26** was 4.8:1 whereas in a previous run the corresponding ratio was 2.9:1. Examination of the *n*-butyllithium used to prepare the LDA showed that it was cloudy, suggesting that lithium hydroxide might have caused the decomposition. To test this hypothesis, the crude product was purified to afford 117 mg (52% recovery) of a mixture of **26** and **32**. This material was epimerized with 1.2 equivalent of LDA (prepared from a new bottle of *n*-butyllithium). HPLC analysis of this crude product showed only a small amount of decomposition, and the ratio of **26** to **32** was 2.6:1. Purification afforded **32** (60 mg, 91% pure) and **26** (25 mg, 89% pure) (73% mass recovery).

Based on the above results, we believe that the scheme shown in Chart 6 will give a better overall radiochemical yield of [^{14}C]artemisinin than the one that we used previously. We plan to start a master run as soon as we receive the radiolabeled starting material, [^{14}C]methyl iodide.

2.7 WR-249309: [^{3}H]Artemisinin; (^{3}H-26)

Work has continued on the synthesis of [^{3}H]-artemisinin [^{3}H]-**26** (Chart 7). In the previous report[1] we reported that hydrogenation of artemisinic acid (**34**) did not occur at ambient temperature and pressure with a commercial sample of cobalt boride as catalyst. This reduction also did not occur with a commercial sample of nickel boride as catalyst. We were, however, able to repeat the reported[3] in situ nickel chloride-sodium borohydride method for conversion of **33** to **34**. Thus, sodium borohydride was added in small portions to a solution of **33** and nickel chloride hexahydrate in methanol. After the evolution of hydrogen had ceased, the mixture was stirred for 1.75 h. Workup afforded a colorless oil (92% yield) whose ^{1}H NMR spectrum indicated it was a diastereomeric mixture of dihydroartemisinic acid (**34**). Analysis by HPLC [Zorbax ODS, 5 μ, 4.6 mm x 250 mm, 80% acetonitrile - 20% water (1% acetic acid), 1.0 mL/min, 210 nm] showed it to be a 4:1 mixture (t_R = 6.2 min, minor; 6.7 min, major diastereomer). Roth and Acton[4] obtained a 5:1 diastereomeric mixture of **34** for this reduction (HPLC[5] system: C18 column, 40% aqueous acetonitrile + 0.1% trifluoroacetic acid, 220 nm: t_R = 14.2 min, minor; 16.4 min, major diastereomer). Because of the success of this reaction we investigated using freshly prepared nickel boride as a hydrogenation catalyst, but first, we wanted to determine if nickel boride was acting as a catalyst with the decomposition of sodium borohydride as a source of hydrogen, or if the reduction of **33** to **34** with this system was due to hydrogen adsorbed/bound to the nickel boride during its preparation, or perhaps to some metal hydride species. Thus, sodium borohydride was added to a solution of nickel chloride hexahydrate in methanol. After the evolution of hydrogen had ceased, the mixture containing the dark clump of nickel boride was sonicated to obtain a fine dispersion and to expel any remaining hydrogen. Compound **33** was added, and the mixture was stirred for 2.3 h. Workup afforded an oil (50% yield) whose ^{1}H NMR spectrum indicated that no reduction had taken place. When the experiment was repeated with absolute ethanol instead of methanol as solvent, a fine black precipitate

Chart 7



a) $^3H_2$, catalyst
b) O_2, methylene blue, hυ
c) air, TFA
d) excess $NaBH_4$, $NiCl_2 \cdot 6H_2O$

formed. After sonication to expel hydrogen, **33** was added and the mixture stirred overnight . Workup in this instance afforded a colorless oil (96% yield) whose ^{1}H NMR spectrum indicated that reduction to **34** had taken place. Whether the longer reaction time or the change in solvent was the cause of the reduction was not determined. In any case, it was apparent that nickel boride prepared this way could reduce **33** to **34** in the absence of hydrogen gas, and thus was unsuitable for carrying out catalytic reductive tritiations where high specific activity was desired. In order to ensure that the nickel boride used as a hydrogenation catalyst did not contain any residual hydrogen from its preparation it was prepared in the following manner. Sodium borohydride was added to a solution of nickel chloride in water. The black precipitate thus formed was isolated, washed first with water and then absolute ethanol and finally heated to dryness under vacuum. Unfortunately, no reduction of **33** was observed when this material was used as a catalyst for hydrogenation. Apparently, the successful conversion of **33** to **34** with the nickel chloride-sodium borohydride system is not due to a catalytic effect of nickel boride with the nascent hydrogen formed from the reaction of sodium borohydride with the solvent.

With these discouraging results, we turned our attention towards using a soluble catalyst for the catalytic hydrogenation of **33** to **34**. Harmon et al.[6] made an extensive investigation of tris(triphenylphosphine)chlororhodium(I) (TPRC) as a catalyst for homogeneous hydrogenation of unsaturated organic compounds. They carried out the hydrogenations in deoxygenated benzene or absolute ethanol at 40-60 °C and 60-100 psi pressure. Even though these conditions are not suitable for catalytic tritiations, we decided to explore the effectiveness of this catalyst for the hydrogenation of **33** to **34** under conditions that could be used for tritiations. We were gratified to find that a clean reduction occurred when an ethanolic solution of **33** and TPRC was left in an atmosphere of hydrogen at ambient temperature and pressure for 2 days. An acid-base workup afforded a semisolid (50% yield) whose ^{1}H NMR spectrum indicated it to be

mostly a single isomer of **34**. The ^{1}H NMR spectrum also indicated that traces of the catalyst were present. Analysis of this product by HPLC indicated that the required isomer (t_R = 6.7 min) was present in a 92:8 ratio. In another experiment, ethanol was removed after reduction and the brownish residue was analyzed by HPLC and ^{1}H NMR. It was found to contain only the reduced product **34** and the catalyst.

Roth and Acton reported[4] that photooxidation of a methylene chloride solution of **34** (0.42 mmol, 5:1 epimer mixture) and methylene blue at -78 °C with a Westinghouse Ceramalux high-intensity C400S51 electric discharge street lamp gave artemisinin in 17% yield. When they carried out the reaction at 0 °C or at room temperature, the yield was half of that obtained at -78 °C. In a subsequent paper[3] Roth and Acton reported a 30% yield of artemisinin when the irradiation of **34** (0.42 mmol) and methylene blue was carried out at 0 °C in acetone for 30 min with the same light source. They obtained an identical result when an acetone solution of **34** (0.42 mmol) and methylene blue was left in direct sunlight for 1 h. The simplicity of the direct sunlight method appealed to us because of the small scale (0.1 mmol) planned for the radiosynthesis.

Thus, an acetone solution containing the crude reduced product of **34** (0.1 mmol), TPRC (left over from the reduction of **33**) and methylene blue was exposed to the sun. Progress of the reaction was monitored by HPLC. When most of the starting material had disappeared (it took 15 h of sunlight for the completion of the reaction), the intermediate hydroperoxide **35** was allowed to air oxidize for 4 days. The crude mixture containing artemisinin was initially purified by a short silica column and then by semi-preparative HPLC. Artemisinin (2.3 mg), thus obtained, was 93% and 89% pure by ^{1}H NMR and HPLC, respectively. Thus the calculated yield of pure artemisinin is only 8%. At this point we were not sure whether or not the TPRC present in the mixture interfered with the photooxidation.

Several experiments were carried out to determine whether or not the presence of TPRC played a role in the low yield of **36**. In the first experiment (entry 1, Table 4) a

solution of **34** (28 mg, 0.094 mmol) and methylene blue (1 mg) in acetone (25 mL) was exposed to sunlight for 6.5 h. A second experiment (entry 2, Table 4) was run simultaneously in which a solution of **34** (22 mg, 0.076 mmol), TPRC (4.7 mg) and methylene blue (1.2 mg) in acetone (25 mL) was also exposed to sunlight for 6.5 h. HPLC analysis of the reaction mixtures showed that the solution without TPRC contained only 2.4% of starting material **34**, whereas the solution with TPRC contained 54% of unreacted **34**. The mixture containing TPRC was left in the hood exposed to fluorescent light for an additional 41 h. HPLC analysis at this time revealed 13% of unreacted **34**. These results indicated that fluorescent light could induce the photochemical reaction, consequently another experiment was done under fluorescent light for comparison. Thus, in a third experiment (entry 3, Table 4) a solution of crude 34 (17 mg, 0.057 mmol) and methylene blue (1.7 mg) in acetone (25 mL) was exposed to fluorescent light for 29 h when HPLC analysis showed that only 8% of unreacted **34** was present. Each of the three experiments were carried through the reaction scheme (Chart 7) to afford artemisinin. Thus, the crude reaction mixtures, after removing methylene blue, were taken up in hexane and left at room temperature after adding a drop of trifluoroacetic acid for the time period specified in Table 4. The final products from the three reactions were purified by chromatography on small silica gel columns and then by semipreparative HPLC. The yields of pure artemisinin were calculated by HPLC using the internal standard method.

The results summarized in Table 4 indicate that a) the presence of TPRC slows the photooxidation step and is detrimental to the final yield of artemisinin (compare entry 2 with 1 and 3), and b) the photooxidation step is slower in the presence of fluorescent light compared to sunlight, but the overall yield of artemisinin is not greatly affected (compare entries 1 and 3).

Table 4

Experiment No.	Percent Unreacted 34 (h)	Reaction Time[b]	Yield of 26 (%)[c]
1	2.4 (6.5, sunlight)	44 h	1.55 mg (5.7)
2	54 (6.5, sunlight)[a]	24 h[a]	0.62 mg (2.9)
3	8 (29, fluorescent)	41 h	1.11 mg (6.8)

[a]13% of unreacted **34** remained after an additional 41 h exposure to fluorescent light. This sample was then used in the next step.

[b]Time for the oxidation-cyclization reaction

[c]Yield percentages were calculated assuming 100% pure starting material **34**.

During the course of this work, we learned from Dr. N. A. Roth that the method described in her patent[7] which bubbles oxygen through the solution and uses a high intensity electric discharge lamp as a source of irradiation is superior to the sunlight method. Consequently we also investigated this method. Thus, 100 mg of pure artemisinic acid (**33**) was reduced with sodium borohydride (200 mg) and nickel chloride hexahydrate (100 mg). Workup afforded an oil whose ^{1}H NMR spectrum showed neither the olefinic proton at 5.12 ppm of **34**, nor the broad singlet at 1.64 ppm attributed to the allylic methyl group of **34**. We assigned structure **36** to this product based on the ^{1}H NMR spectrum. HPLC conditions employed for the analysis of dihydroartemisinic acid (**34**) did not separate the tetrahydrohydroartemisinic acid (**36**) from **34**. Careful reanalysis of the ^{1}H NMR spectra of the reduction products of **33** from previous runs by the sodium borohydride-nickle chloride method showed them to contain various amounts of the tetrahydro compound **36**. Only the catalytic reduced product was free of any over reduced compound.

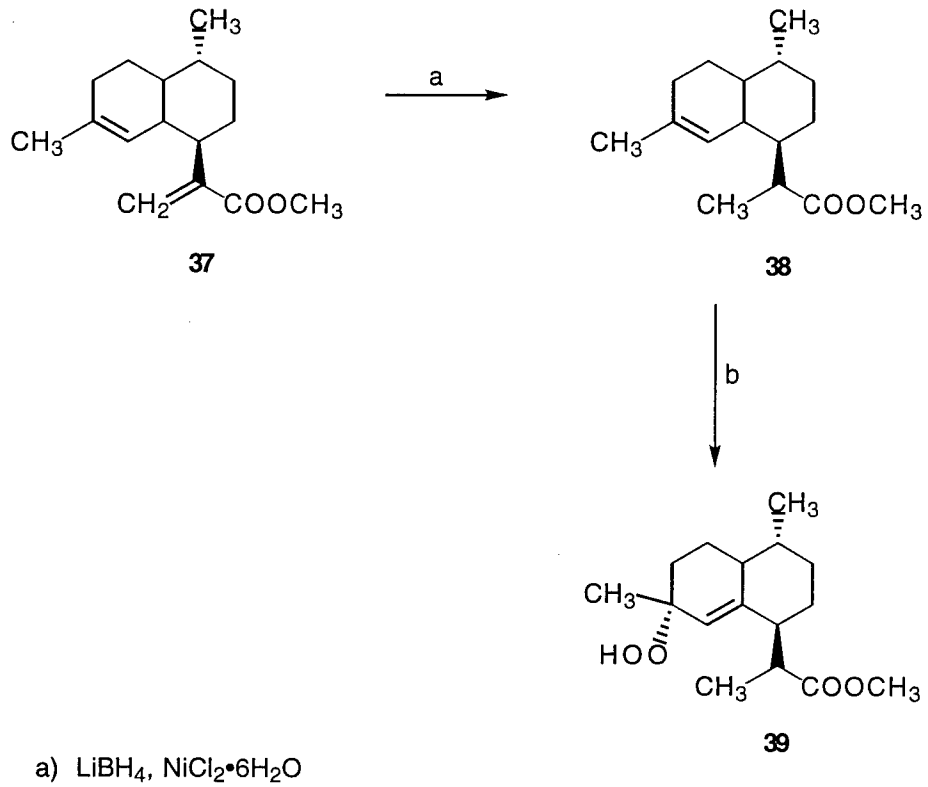
Jung *et al* .[8] reported quantitative conversion of **33** to **34** by reduction with lithium borohydride (5.3 equiv.) and nickel chloride (0.49 equiv.). Thus, a methanolic solution of **33** (23.4 mg, 0.1 mmol) and nickel chloride hexahydrate (12 mg, 0.049 mmol) was

stirred with lithium borohydride (12.3 mg, 0.53 mmol) for 2 h at room temperature. Workup afforded an oil (24 mg) whose ^{1}H NMR spectrum showed 12% of unreacted starting material **33** present. No tetrahydroartemisinic acid (**34**) was present in this oil. This oil was used without further purification for the experiment described below.

In Roth's procedure[7], irradiation of **34** was done in acetone at 0 °C. Jung *et al*.[8] obtained a 70% isolated yield of intermediate hydroperoxide ester **39** from irradiating a methanolic solution of dihydroartemisininic acid methy ester (**38**) at room temperature (Chart 8). We decided to investigate the room temperature photooxidation reaction because it would be simpler experimentally for the radiosynthesis. Thus an acetone (20 mL) solution of crude dihydroartemisinic acid (24 mg) and methylene blue (1.7 mg) was irradiated with a high intensity halogen discharge lamp while continuously bubbling oxygen through the reaction mixture for 2 h. HPLC analysis at this time showed only a trace of starting material. The residue, after removing methylene blue, was treated with hexane (10 mL) and a drop of trifluoroacetic acid and left at room temperature for four days. The crude product was purified on a short silica column to afford 4.7 mg (18%) of colorless solid whose ^{1}H NMR spectrum was identical to that of standard artemisinin except for a singlet at 1.58 ppm which was not present in the spectrum of standard material. HPLC analysis of this product showed it to be 87% pure.

In order to optimize the conditions for the radiolabeled synthesis, three more experiments were carried out. The results are summarized in Table 5. For each experiment, the photooxidation was carried out by irradiating a solution of **34** and methylene blue with a high intensity halogen lamp under a stream of oxygen at the temperature indicated in the table until all of the **34** had been consumed. The crude **35** was then dissolved in hexane containing a drop of trifluoroacetic acid and stored under an oxygen atmosphere at ambient temperature for 5 days. The reactions were then worked up and the crude **26** purified by chromatography. The third experiment was identical to the first

Chart 8



a) $LiBH_4$, $NiCl_2 \cdot 6H_2O$
b) $^1O_2$, methylene blue, methanol

except that **34** was contaminated with the reduction catalyst TPRC. The results from these experiments indicated that the photooxidation can be done at ambient temperature rather than 0 °C (compare runs 1 and 2), and that although the presence of TPRC slowed the photo oxidation reaction, it did not appreciably affect the yield of **26** (compare runs 1 and 3) under these conditions.

Next we investigated the reproducibility of the third experiment in Table **5** since it will be the easiest to carry out with radiolabeled material. We found that this reaction was reproducible. A 15% yield of artemisinin (96% pure) was obtained from 0.1 mmol of **33**.

Next a tracer experiment was conducted using deuterium as the label. Thus, a mixture of **33** (22.8 mg, 0.1 mmol) and TPRC (4.4 mg, 0.005 mmol) in absolute ethanol

Table 5

Run	34	Temperature	Time of irradiation	Yield of 26	(Purity)
1	0.1 mmol	r.t.	2 h	12.6%	95%
2	0.1 mmol	0 °C	2 h	10.6%	91%
3	0.1 mmol	r.t.	6.25 h	11.7%	96%

(2.5 mL) was stirred under a deuterium atmosphere at ambient temperature and pressure overnight. Analysis of the reduction mixture by HPLC and ^{1}H NMR showed that no reduction had taken place. We were concerned that this failure might be due to an isotope effect, since an even greater isotope effect would be expected in the tritiation reaction. The deuterium reaction was repeated, and after stirring overnight HPLC analysis indicated partial reduction had occurred. It was noted that the room temperature varied from 20-23 °C during this reaction and that the catalyst had turned black. This mixture was kept at 27 °C and after again stirring overnight, HPLC analysis indicated an additional small amount of reduction had taken place. To determine if an isotope effect was the cause of the slow reduction, the deuterium was replaced with hydrogen and the reaction allowed to continue at 27 °C overnight. HPLC analysis at this time showed no further reduction had taken place, suggesting that something other than an isotope effect was the cause of the inconsistency of this reaction. We investigated the effect of two variables on the reduction, namely, temperature and the amount of catalyst. In the first experiment, a mixture of **33** (22.8 mg, 0.1 mmol) and TPRC (4.4 mg, 0.005 mmol) was stirred under deuterium at 27 °C overnight. A black precipitate was observed and the analysis of the dark mixture showed that there was only partial reduction. The next experiment was done under identical conditions except that the amount of TPRC was increased to 8.8 mg (0.01 mmol). After stirring overnight, the

mixture had turned orange-brown but there was no black precipitate. Analysis of this mixture by HPLC showed that it was fully reduced. ^{1}H NMR of this mixture also confirmed that there was complete reduction to **34**. The crude **34** (30 mg) was dissolved in acetone (20 mL) containing methylene blue (1.9 mg) and irradiated with a high intensity halogen discharge lamp for 4 h 45 min while continuously bubbling oxygen through the solution. The residue, after removing methylene blue, was treated in hexane (10 mL) with two drops of trifluoroacetic acid and left at room temperature in an oxygen atmosphere for 5 days. The crude product was purified on short silica column to afford 3.6 mg (12.7%) of colorless solid whose ^{1}H NMR indicated that deuteration had occurred. Mass spectrometry (CI, NH_3-CH_4) of this material showed molecular ions at *m/z* 302 ($M+NH_4$) and 285 (M+H). There was no evidence of proton exchange alpha to the lactone function.

We next looked for reliable conditions to carry out this reaction at ambient temperature which would be advantageous for the tritiation experiment. We also investigated reducing the time required for the oxidation-ring closure reaction (step c, Chart 7) to see if the isolated yield of artemisinin could be improved. We were gratified to find that by doubling the amount of the catalyst TPRC from 8.8 mg to 17.6 mg the complete deuteration could be achieved at 20 °C. Thus a mixture of artemisinic acid (22.7 mg, 0.1 mmol), and TPRC (17.6 mg, 0.02 mmol) in absolute ethanol (2.5 mL) was stirred under deuterium at 20 °C overnight. Analysis of this mixture by HPLC showed that it was fully reduced. The crude **34** was dissolved in acetone (20 mL) containing methylene blue (2.4 mg) and irradiated with a high intensity halogen discharge lamp for 3 h. (Note: In the previous run, this irradiation took 4 h 45 min. The difference between this run and the previous one was the amount of methylene blue used; 2.4 mg vs 1.9 mg). The residue, after removing methylene blue, was treated in hexane (10 mL) with two drops of trifluoroacetic acid and left at room temperature in an oxygen atmosphere for 68 h (Note: In the previous run, this step was carried out for 5 days). The crude product

was purified on silica column to afford 5.9 mg (21.3%) dideuteroartemisinin whose ^{1}H NMR was identical to the one from the previous run.

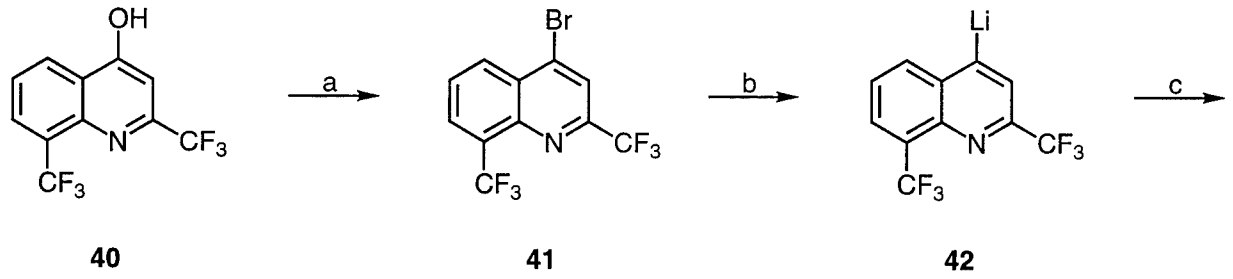
The next run was carried out under identical conditions to the one described above except that the last step was done only for 17 h. In this case only a slightly improved yield (6.9 mg, 25%) of dideuteroartemisinin was obtained. The HPLC purity of the final product from these two runs was 95%. We believe that the scheme is now sufficiently reliable that a master run can be planned for the future.

2.8 WR-142490: [Methanol-^{14}C]Mefloquine (46)

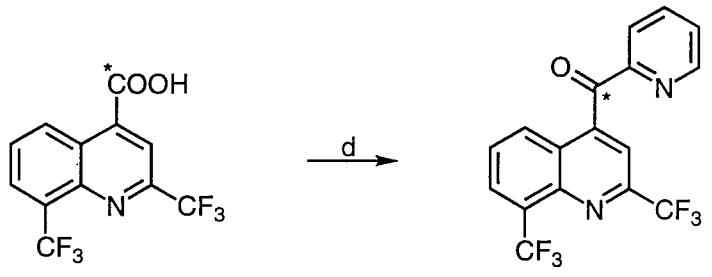
Work has also continued on the synthesis of [methanol-^{14}C]mefloquine ([^{14}C]-**46**) by the scheme shown in Chart 9. Reaction of 2,8-bistrifluoromethyl-4-quinolinol (**40**) with phosphorus tribromide gave a quantitative yield of 4-bromoquinoline **41**.[9] Initial attempts to prepare carboxylic acid **43** via the literature procedure[9] were not successful. The major problem appeared to be generation of lithium salt **42** since 60-80% of bromo-quinoline **43** was recovered from these reactions. Taking special precautions to dry the reaction solvents, changing the solvent from ether to THF and replacing *n*-butyllithium with *tert*-butyllithium to generate **42** did not significantly improve the reaction. A nearly quantitative formation of **42** was achieved though, by addition of the *n*-butyllithium solution to an ethereal solution of **41**; the reverse order of addition from that of the literature.[9] Carbonation of this solution on the vacuum line however, afforded only a 46% yield of carboxylic acid **48**.

Future studies will focus on increasing the yield of the carbonation reaction since carbon dioxide will be the source of the radiolabel. We plan to do experiments with carbon dioxide as the limiting reagent, and also reduce the size of the reaction flask to increase the carbon dioxide partial pressure.

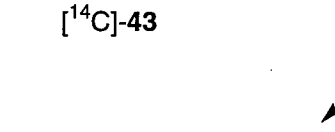
Chart 9

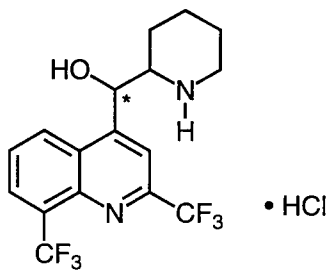


40 **41** **42**



[^{14}C]-**43** [^{14}C]-**45**



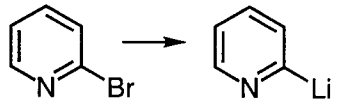


[^{14}C]-**46**

a. $POBr_3$

b. n-BuLi

c. $^{*}CO_2$ (from $Ba^{*}CO_3 + H_2SO_4 \rightarrow {}^{*}CO_2$)

d. n-BuLi + 

44

e. H_2, Pt

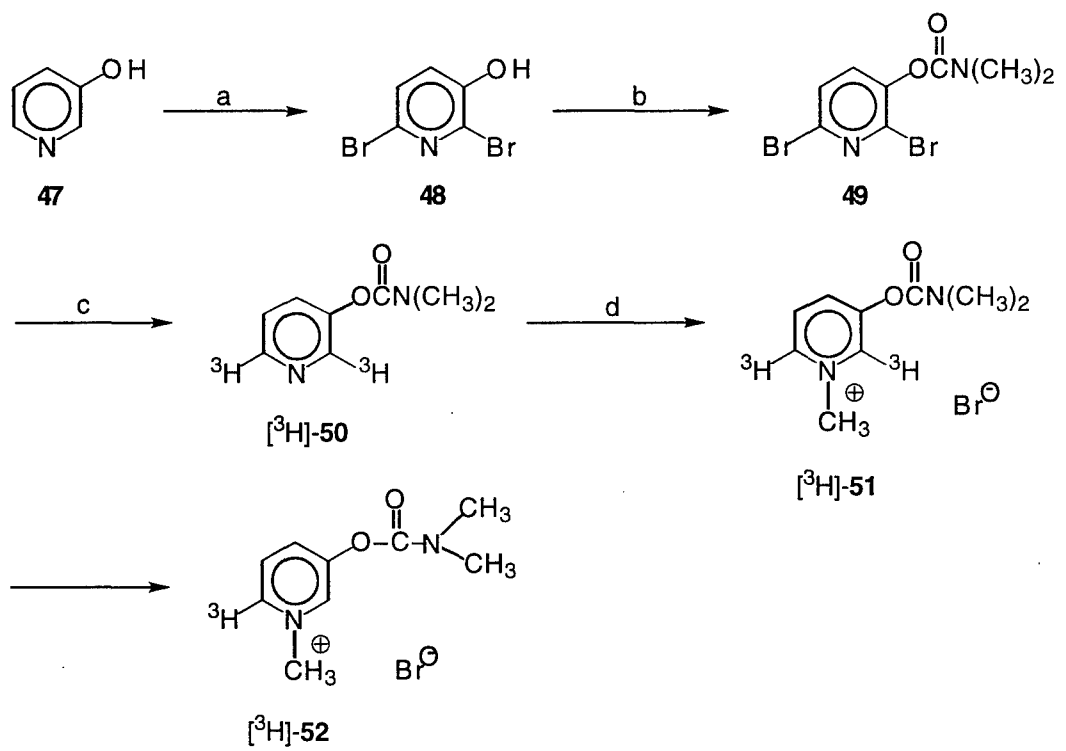
f. HCl

2.9 WR-250710: [^{3}H]Pyridostigmine Bromide (52)

A resynthesis of [6-^{3}H]pyridostigmine bromide ([^{3}H]-**52**, Chart 12) was started this report period. Tritium reduction of dibromide **49** gave tritium labeled **50** as expected, but attempted quarternization of [^{3}H]-**50** with methyl bromide did not give [^{3}H]-**51** as cleanly as anticipated.

An unknown radioactive impurity was formed during the reaction. A second attempt at resynthesis also gave this impurity. We have not been able to identify or remove this impurity to date. At present, we are doing some test reactions with non-radioactive materials, as well as some ^{3}H NMR studies to determine the identity of the impurity, with the expectation that the information will give some insight as how to remove the impurity or avoid its formation.

Chart 10



a) Br_2, pyridine
b) $(CH_2)_2NCOCl$
c) T_2, Pd/C, MgO, EtOH
d) CH_3Br, acetone
e) exchange in CH_3OH

3.0 Purifications

3.1 WR-250710: [2-^{14}C]Pyridostigmine Bromide

Prior to shipment [2-^{14}C]WR-250710 was analyzed and found to be impure. Attempted purification by crystallization from ethanol-ether gave material of only marginally improved purity. Preliminary work indicated that the material could be purified by chromatography on alumina. An 80 mg sample of [2-^{14}C]pyridostigmine bromide which was ~88% radiochemically pure was chromatographed on basic alumina using a step gradient from 15% methanol-ethyl acetate to 60% methanol-ethyl acetate to afford 34 mg of material which was 98% radiochemically pure. Unfortunately attempts to repeat this chromatographic purification was unrewarding. The original purification was done with a two-year old batch of alumina, and attempts to repeat it with new batches of alumina from several different sources which have deactivated by a variety of procedures have not been successful. Further work on this purification is being carried out as time allows.

3.2 WR-243251: 7-Chloro-3-(2,4-dichlorophenyl)-1-{[3-(dimethylamino)propyl]-amino}-1,2,3,4-tetrahydro-9(10H)-[9-^{14}C]acridone

A purity check of [^{14}C]WR-243251 showed that it was only 88% radiochemically pure. Attempted purification by crystallization was not successful. A 50 mg sample from the recrystallization attempts was chromatographed on basic alumina using a step-gradient of ethyl acetate to 10% methanol-ethyl acetate. This afforded 36 mg of material that was 96% radiochemically pure. This successful purification was accomplished with the two-year old batch of alumina described above, and could not be repeated with new batches of alumina even with deactivation. Further work on this purification is being carried out as time allows.

3.3 WR-242511: 8-[(4-Amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl[^{14}C]quinoline DL-Tartrate

Analysis of the title compound (lot no LF-7870-2) by HPLC-RAM showed that it was 93% radiochemically pure. The material was converted to its free base prior to purification by flash chromatography [20 mm id column, 170 mm of silica gel, 40% methanol-ethyl acetate (200 mL), 60% methanol-ethyl acetate (200 mL), 60% methanol-ethyl acetate with 1% TEA until the product eluted]. After making the tartrate salt, a total of 7.9 mCi of material was isolated that was 97.4% radiochemically pure (HPLC-RAM) with specific activity of 13.1 mCi/mmol (25.0 μCi/mg).

4.0 Shipments

A total of nine shipments (Table 6) were made to authorized investigators during the period November 15, 1995 to November 14, 1996 as directed by the Project Officer.

5.0 Inventory

A list of the compounds held in inventory on December 1, 1996 by the Research Triangle Institute for USAMRDC is given in Table 7.

6.0 Priority List

The compounds that are currently assigned to us and their priorities are listed in Table 8.

Table 6

SHIPMENTS

November 15, 1995 - November 14,1996

WR No.	Name	Lot No.	Amount	Date	Recipient
1065	2-[(3-Aminopropyl)amino][1,2-^{14}C]-ethanethiol Dihydrochloride	5172-103	0.020 mCi	04/16/96	Dr. John L. A. Mitchell N. Illinois Univ., DeKalb, IL
255663	[16-^{14}C]Artelinic Acid	CT-8440-43	0.505 mCi	04/16/96	Dr. James Peggins WRAIR
171669	1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N,N-di-n-butylamino)-[1-^{14}C]propyl]phenanthrene Hydrochloride	CF-8144-182-3	250 µCi	05/28/96	Dr. Kaveh Zamani WRAIR
250710	[2-^{14}C]Pyridostigmine Bromide	CT-4167-127	4.1 mCi	06/11/96	Dr. James Peggins WRAIR
254986	[16-^{14}C]Artemether	CT-8440-77-1	1.5 µCi	07/09/96	Dr. James Peggins WRAIR
178460	1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N-n-butylamino)-[1-^{14}C]-propyl]phenanthrene Hydrochloride	CF-8448-129-4	0.5 mCi	07/30/96	Dr. James Peggins WRAIR
242511	8-[(4-Amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl-[4-^{14}C]quinoline (DL)-Tartrate	LF-7870-2	5 mCi	08/26/96	Dr. James Peggins WRAIR
1439	2,2'-Dichloro-N-methyldi([1-^{14}C]-ethyl)amine Hydrochloride	CT-8136-181-7	1.018 mCi	09/03/96	Major Marino WRAIR

Table 6 (continued)

SHIPMENTS

November 15, 1995 - November 14,1996

WR No.	Name	Lot No.	Amount	Date	Recipient
250710	[6-^{3}H]Pyridostigmine Bromide	CT-4537-81	10.1 mCi	10/01/96	Dr. Kathleen Leo WRAIR

Table 7

RESEARCH TRIANGLE INSTITUTE

Inventory - Contract No. DAMD17-93-C-3001

December 1, 1996

WR No.	Compound	Lot No.	Origin	Specific Activity mCi/mmol	Amount Available mCi
1065	2-[(3-Aminopropyl)amino][1,2,-^{14}C]-ethanethiol Dihydrochloride (CT-7242-85)	5172-103	RTI	24.4	0.533
		5662-81-B	RTI	10.5	0.508
		5662-161	RTI	9.89	0.53
1439	2,2'-Dichloro-N-methyldi([1-^{14}C]ethyl)amine Hydrochloride	CT-8136-181-3	RTI	17.0	4.51
1439	2,2'-Dichloro-N-methyldi([1-^{14}C]ethyl)amine Hydrochloride	CT-8136-181-7	RTI	17.0	1.814
1544	[quinoline-3-^{14}C]Chloroquine Diphosphate	CT-8136-3-1	RTI	20.2	3.136
2721	S-[2-(3-Aminopropylamino)[1,2-^{14}C]ethyl]-phosphorothioic Acid	CT-5172-96	RTI	99 µCi/mg	1.58
2823	S-[2-(5-Aminopentylamino)[1,2-^{14}C]ethyl]-phosphorothioic Acid	3612-95	RTI	33.3 µCi/mg	0.83
+2975	[quinoline-2,4-^{14}C]Primaquine Diphosphate	2850-51-E	New England Nuclear	1.55	0.471
		2176-067	New England Nuclear	2.57	9.485
+2978	[2-^{14}C]Pyrimethamine	2572-194	Amersham Corp.	14.7	0.75
		3193-158	Amersham Corp.	54	25.5
3689	S-[2-(3-Methylaminopropylamino)[1-^{14}C]-ethyl]phosphorothioic Acid	CT-4928-123-1	RTI	54.6 µCi/mg	2.69
		CT-4928-127-1	RTI	46.1 µCi/mg	1.476
		CT-5385-115	RTI	51.3 µCi/mg	10.77
6026	6-Methoxy-8-(6-diethylaminohexylamino)-[2,3-$^{2}H_2$]lepidine Dihydrochloride	CT-4928-79	RTI	N/A	517 mg
6026	6-Methoxy-8-(6-diethylaminohexylamino)-lepidine-4-^{14}C Dihydrochloride	CT-5385-99-1	RTI	16.1	2.39
		CT-5385-99-2	RTI	16.2	1.04
6241	[3-^{14}C]Atropine Sulfate Monohydrate	4869-147-3	RTI	13	1.8

WR No.	Compound	Lot No.	Origin	Specific Activity mCi/mmol	Amount Available mCi
6570	[carbamate methyl-^{14}C]Physostigmine Salicylate	MM-5241-102-A MM-5241-102-B	RTI RTI	55 17.6	1.0 1.0
6570	[2-^{14}C]Physostigmine	CT-5324-55	RTI	28.6	10.4
6570	(±)-[1 methyl-$^{2}H_3$-2,2,3,3-$^{2}H_4$]-Physostigmine Salicylate	MM-5616-131	RTI	N/A	211.0 mg
6570	[benzene ring-^{3}H]Physostigmine	TRQ-4569	Amercham Corp.	16,000	3.73
16411	2-[(Hydroxyimino)methyl]-1-([^{14}C]methyl-pyridinium Chloride	4929-61-A	RTI	1.9	3.66
30090	2-(3,4-Dichlorophenyl)-6,8-dichloro-[2-^{14}C]-quinolyl]-4-dibutylaminomethylcarbinol Hydrochloride	302-4a*	Monsanto	5.43	2.78
40070	2,4-Diamino-5-piperonyl[2-^{14}C]pyrimidine	146*	Monsanto	10.6	5.85
46234	5-Chloro-2-hydroxy-N-(2-chloro-4-nitro-phenyl)[ring-U-^{14}C]benzamide	5513-153-B 5513-166	RTI RTI	18.0 9.04	2.52 0.828
46234	5-Chloro-2-hydroxy-N-(2-chloro-4-nitro-[U-$^{13}C_6$]phenyl)benzamide	5662-21	RTI	N/A	161 mg
99210	4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2,4,5-trichlorophenoxy)-propyloxyl-s-[2-^{14}C]triazine Hydrochloride	346a*	Monsanto	9.53	1.84
142490	Erythro-α-(2-piperidyl)-2,8-bis(trifluoro-methyl)-4-quinoline[^{14}C]methanol Hydrochloride	411a* 3793-133	Monsanto RTI	11.5 57.8	2.36 3.28
142490	Erythro-α-(2-piperidyl)-2,8-bis(trifluoro-methyl)-4-quinoline[^{14}C]methanol Methane-sulfonate	433a*	Monsanto	11.6	1.51

WR No.	Compound	Lot No.	Origin	Specific Activity mCi/mmol	Amount Available mCi
149024	1,18-Diamino-6,13-diaza-9,10-dithia-[7,8,11,12-^{14}C]octadecane Tetrahydrochloride	3612-55	RTI	13.5	.94
151327	S-[3-(3-Methylaminopropylamino)[1-^{14}C]-propyl]phosphorothioic Acid	CT-5385-161-1 CT-5324-179	RTI RTI	105 µCi/mg 110 µCi/mg	7.56 2.519
169626	4,6-Diacetamido-1,2-dihydro-2,2-dimethyl-1-[γ-(2',4',5'-trichlorophenoxy)-propyloxy]-s-[2-^{14}C]triazine	CT-3652-93-1	RTI	16.3	8.16
171669	1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N,N-di-n-butylamino)[1-^{14}C]propyl]-phenanthrene Hydrochloride	CF-8144-182-3	RTI	29.8	19.25
172435	3-Di-n-butylamino-1-[2,6-bis(trifluoromethylphenyl)-4-pyridyl][1-^{14}C]propanol Hydrochloride	348b*	Monsanto	10.2	1.34
172435	3-Di-n-butylamino-1-[2,6-bis(trifluoromethylphenyl)-4-pyridyl][1-^{14}C]propanol Methanesulfonate	2850-127	RTI	11.5	1.07
177602	Threo-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline[^{14}C]methanol Hydrochloride	469a 469-2a*	Monsanto Monsanto	13.4 13.4	3.30 1.80
177602	Threo-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline[^{14}C]methanol Methanesulfonate	434a*	Monsanto	11.9	0.95
178460	1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N-n-butylamino)[1-^{14}C]propyl]phenanthrene Hydrochloride	3793-91 CF-8448-129-4	RTI RTI	16.0 36.4	1.17 15.5
180117	α-(2-Piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridine[^{14}C]-methanol Hydrochloride	365-2c*	Monsanto	26 µCi/mg	2.16

WR No.	Compound	Lot No.	Origin	Specific Activity mCi/mmol	Amount Available mCi
180117	α-(2-Piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridine[^{14}C]-methanol Phosphate	443-2a* 443-2b* 536c*	Monsanto Monsanto Monsanto	10.6 10.3 35 μCi/mg	3.87 2.81 2.59
180409	Threo-α-(2-piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridine-[^{14}C]-methanol Phosphate	536a	Monsanto	19.8	4.018
184806	2,8-Bis(trifluoromethyl)-4-(1-hydroxy-3-N-t-butylamino[1-^{14}C]propyl)quinoline Phosphate	385-2b* 2850-25	Monsanto RTI	16.5 μCi/mg 11.2	1.2 1.5
194965	4-[^{14}C]t-Butyl-6-t-butylaminomethyl-2-(4-chlorophenyl)phenol Phosphate	3612-151	RTI	20.9	0.65
225448	8-(4-Amino-1-methylbutylamino)-6-methoxy-5-(3-trifluoromethylphenoxy)-[4-^{14}C]quinoline Succinate	CT-2575-191	RTI	12.5	16.45
226253	Erythro-α-(2-piperidyl)-2-trifluoromethyl-6,8-dichloro-4-quinoline[^{14}C]methanol Methanesulfonate	2572-114 2572-157	RTI RTI	33 10	6.643 8.52
228258	4'-Chloro-5-[(7-chloro-4-[4-^{14}C]quinolyl)-amino]-3-[(1,1-dimethylethyl)amino]-methyl][1,1'-biphenyl]-2-ol Dihydrochloride	CT-3181-17-1 CT-3183-17-2	RTI RTI	20.4 20.2	19.07 2.10
238605	8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)-phenoxy][4-^{14}C]quinoline Succinate	CT-6949-61	RTI	21	11.625
238605	8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)-[6-^{3}H]phenoxy]quinoline Succinate	CT-6949-145	RTI	165	78.99
242511	8-[(4-Amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl[4-^{14}C]-quinoline (DL)-Tartrate	LF-7690-61	RTI	17	2.34

WR No.	Compound	Lot No.	Origin	Specific Activity mCi/mmol	Amount Available mCi
242511	8-[(4-Amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl[4-^{14}C]-quinoline (DL)-Tartrate	LF-7870-2	RTI	13.1	2.9
243251	7-Chloro-3-(2,4-dichlorophenyl)-1-{[3-(dimethylamino)propyl]imino}-1,2,3,4-tetrahydro-9(10H)[9-^{14}C]acridone	CT-7861-93-1	RTI	57.2	111.60
249309	[16-^{14}C]Artemisinin	8160-109-7	RTI	28.8	1.1
249655	1-(2-Hydroxyimino[^{14}C]methyl)-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxapropane Dichloride Monohydrate ([^{14}C]HI-6•H_2O)	MA-7082-148	RTI	5.96	1.467
250165	[6-^{14}C]Allopurinol Riboside	CT-3892-91-1	RTI	7.85	0.48
250710	[N,N-dimethylamino-$^{2}H_6$]Pyridostigmine Bromide	3959-195	RTI	N/A	0.4 g
250710	[2-^{14}C]Pyridostigmine Bromide	CT-4167-127	RTI	18.0	0.0
250710	[6-^{3}H]Pyridostigmine Bromide	CT-4537-81	RTI	22,500	138.15
250710	[carbamate methyl-^{14}C]Pyridostigmine Bromide	CT-5385-67	RTI	37.6	66.96
253997	[10-^{3}H]Dihydroartemisinin	CT-7242-175	RTI	13,000	149.53
254986	[16-^{14}C]Artemether	CT-8440-77-1	RTI	28.6	0.37
255131	[ethyl-$^{2}H_5$]β-Arteether	5513-181-D	RTI	N/A	1.04 g
255131	β-[16-^{14}C]Arteether	CT-8136-109	RTI	28.8	1.10
255663	[16-^{14}C]Artelinic Acid	LF-7044-94	RTI	13	0.287
255663	[16-^{14}C]Artelinic Acid	CT-8440-43	RTI	32.4 μCi/mg	0.825
269410	p-Aminoheptano[U-^{14}C]phenone	CT-7442-55-1	RTI	17.2	24.95
	Ethyl Di([$^{2}H_3$]methyl)aminophosphoramidic Acid Sodium Salt	LF-7690-187	RTI	N/A	2.69 g

WR No.	Compound	Lot No.	Origin	Specific Activity mCi/mmol	Amount Available mCi

*Purity of these compounds have not been checked at RTI. Specific activity and amount available for shipment are those stated by the originating source and have not been confirmed at RTI.
+WHO compounds.
#Actual value of the specific activity will be less depending on length of storage due to the relatively short half life of ^{3}H.
≠This sample was impure when last checked.

Table 8

WRAIR PRIORITY LIST

Revised December 1, 1996

Priority	WR Number	Name	Amount (Specific Activity)	Comment	Estimated Completion Date[a]
1	250710	[^{3}H]Pyridostigmine Bromide	10-15 mCi (high specific activity)	Master Synthesis started	
2	249309	[^{3}H]Artemisinin	10-15 mCi (high specific activity)	Development work started	
3	249309	[^{14}C]Artemisinin	15-20 mCi (15-20 mCi/mmol)	Repeat Synthesis to be started	
4	242511	8-[(4-Amino-1-methylbutyl)-amino]-5-(1-hexoxy)-6-methoxy-4-methyl[4-^{14}C]-quinoline (DL)-Tartrate	15-20 mCi (15-20 mCi/mmol)		
5	243251	7-Chloro-3-(2,4-dichlorophenyl)-1-{[3-(dimethylamino)propyl]-imino}-1,2,3,4-tetrahydro-9-(10H)[9-^{14}C]acridone	30-40 mCi (15-20 mCi/mmol)		
6	238605	8-[(4-Amino-1-methylbutyl)-amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy]-[4-^{14}C]quinoline Succinate	15-20 mCi (20-25 mCi/mmol)		
7	1544	[quinoline-3-^{14}C]Chloroquine	15-20 mCi (20-25 mCi/mmol)		
8	2975	[quinoline-2,4-^{14}C]Primaquine	10 mCi (10-15 mCi/mmol)		
9		Tetraoxane compound	10-15 mCi (10-15 mCi/mmol)		
10	142490	Mefloquine	60 mCi (25-30 mCi/mmol)	Development work started	
11	171669	Halofantrine	25 mCi (25-30 mCi/mmol)		

Table 8 (continued)

WRAIR PRIORITY LIST

Revised December 1, 1996

Priority	WR Number	Name	Amount (Specific Activity)	Comment	Estimated Completion Date[a]
12	178460	Desbutylhalofantrine	25 mCi (25-30 mCi/mmol)		
13	255131	[^{14}C]Arteether	1-5 mCi (15-20 mCi/mmol)	Repeat Synthesis to be started upon request	
14		[ethyl-2-^{3}H]CEES	25-50 mCi (100 mCi/mmol)	Development work started	on hold
15		[$^{2}H_8$]Thiodiglycol	3 g	To be prepared upon request	
16		[^{14}C]Perfluoroisobutylene	15 mCi (15 mCi/mmol)	Development work started	on hold
17		Bis(trifluoromethyl)disulfide	15 mCi (15 mCi/mmol)	Development work completed	on hold

[a]This date is only a gross estimate and may be several months off in either direction.

7.0 References

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APPENDIX

Synthesis Report

Walter Reed Institute of Research

Contract DAMD17-93-C-3001

2,2'-Dichloro-N-methyldi([1-^{14}C]ethyl)amine Hydrochloride

WR-1439

Lot No. CT-8136-181-3

November 1995

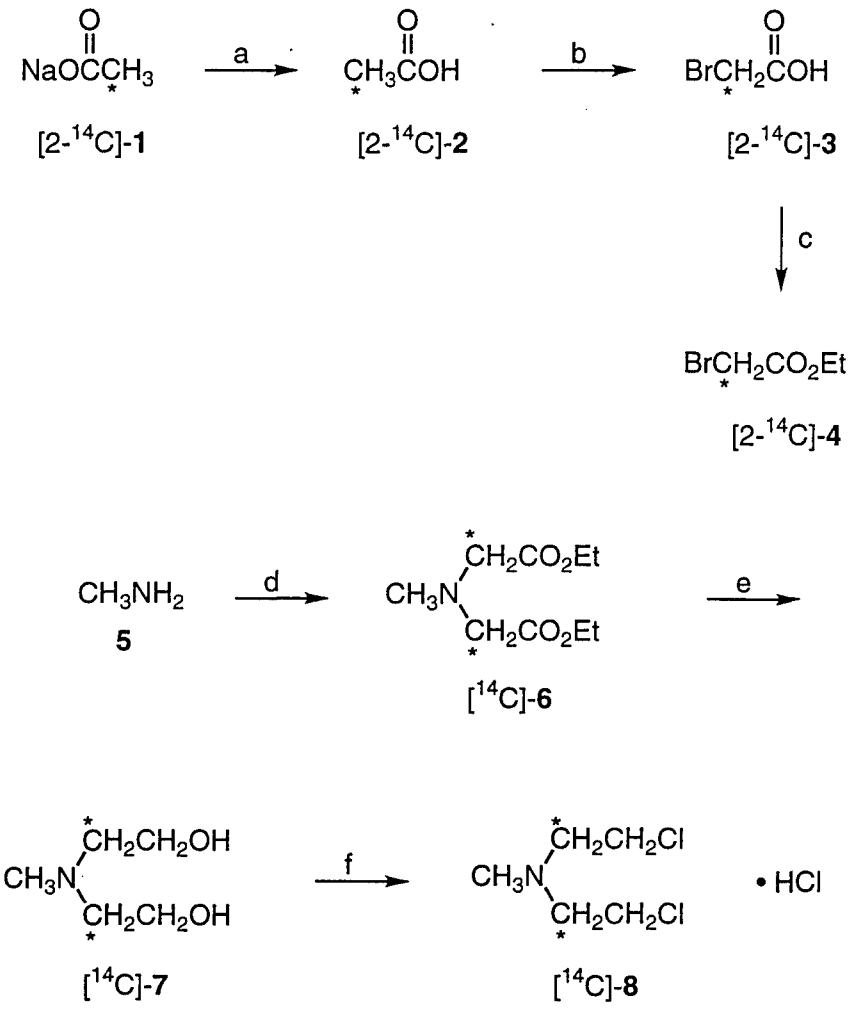
Charles Twine
J. A. Kepler

Research Triangle Institute
Research Triangle Park, NC 27709

Experimental

Analytical TLC were performed using E. Merck silica-gel F-254 and RP-18 F-254 plates. Column chromatographies were performed with E. Merck silica gel 60 (230 - 400 mesh). Solvents were removed from solutions on a rotary evaporator under water aspirator vacuum and ambient temperature unless otherwise noted. Radioactive samples were counted on a Packard Tri-carb 4000 liquid scintillation spectrometer in Ultima Gold cocktail. Developed TLC plates were scanned on a Berthold model LB 283 Linear Analyzer system. HPLC was done using Waters Associates Model 6000A dual pump system with a Model U6K septumless injector and a Berthold Model LB503-HDS radioactivity monitor as the detector.

Chart 1



a) H_3PO_4, P_2O_5
b) $(CF_3CO)_2O$, Br_2
c) EtN_2
d) [^{14}C]-**4**, Et_3N
e) $LiAlH_4$
f) $SOCl_2$

[2-^{14}C]Acetic Acid ([2-^{14}C]-2)

Sodium [2-^{14}C]acetate ([2-^{14}C]-**1**) (155 mg, 1.89 mmol, 56.0 mCi/mmol, 106 mCi) was weighed into a 25 mL recovery flask. The spatula was rinsed with sodium hydroxide solution (~ 0.5 mL, 0.1 N) and this added to the flask. The resulting solution was freeze-dried on the high vacuum manifold. The residue was heated to ~ 80 °C with the heat gun for 3 min and then allowed to cool. The resulting solid was allowed to stand overnight under vacuum (0.01 Torr) while connected to a second flask containing phosphorus pentoxide.

The flask containing [2-^{14}C]-**1** was removed from the manifold and phosphoric acid (100%, 6.5 mL) was added. The flask was rotated to ensure all particles of [2-^{14}C]-**1** were coated with acid. The flask was reattached to the manifold and the pressure stepped down to 0.01 Torr. The reaction flask was heated with an oil bath to 125 °C over ~ 30 min and then held at this temperature for an additional 1.5 h while collecting the distillate in a second, tared flask cooled with liquid nitrogen. The reaction flask was cooled to room temperature and unlabeled acetic acid (**2**) (57 μL, 1.0 mmol) was added. The flask was reattached to the manifold and the pressure stepped down to 0.01 Torr as before. This flask was then heated to 125 °C and held at this temperature for 1 h.

The receiver flask was warmed to room temperature and then carefully removed from the manifold. The distillate of [2-^{14}C]-**2** weighed 192 mg (3.2 mmol). This corresponds to a yield of 110%. It is possible some water distilled out of the phosphoric acid and added to the weight of [2-^{14}C]-**2**. This was used as is for the next step.

Chemicals and Sources

Sodium [2-^{14}C]Acetate	Dupont NEN NEC-085H	Lot no. 2967-492SP
Acetic Acid	Fisher A38-212	Lot no. 945572
Phosphoric Acid (85%)	Baker 5-0262	Lot no. 116852
Phosphorus Pentoxide	Aldrich 29,892-0	Lot no. 945572

Ethyl [2-^{14}C]Bromoacetate ([2-^{14}C]-4)

Trifluoroacetic anhydride was added dropwise over 2 min to the flask containing the crude [2-^{14}C]-**2** (192 mg) at 0 °C. The resulting solution was stirred for 15 min, and then bromine (522 mg, 3.26 mmol, 168 μL) was added. The resulting solution was tightly stoppered and stirred at room temperature for 42 h. The resulting pale red solution was cooled to 0 °C and hydrolyzed by the slow addition of water (203 mg, 11.3 mmol, 203 μL). This solution was stirred for 1 h and then stripped to remove the trifluoroacetic acid. The residue was mixed with hexane (3 mL) and stripped. This was repeated a total of three times. The resulting off-white solid was dissolved in ethyl ether and treated with excess diazoethane. This yellow solution was allowed to stand overnight.

The solution was counted (63.2 mCi) and then distilled down to a volume of ~ 1 mL using a short path still head on a packed column. The column was rinsed with ~ 3 mL of ethyl ether. The flask containing the product in ~ 4 mL of ethyl ether was attached to the vacuum manifold. The ethyl ether was distilled out at 0.01 Torr with the pot at -4 °C and the receiver at -77 °C. The product was then distilled with the pot at 35-40 °C and the receiver cooled with liquid nitrogen to yield 318 mg (1.90 mmol, 66% yield) of colorless oil. This was used as is for the next reaction.

Chemicals and Sources

Trifluoroacetic Anhydride	Aldrich 10,632-2	Lot no. 03907 AV
Bromine	Aldrich 20,788-8	Lot no. 06014 LZ
Hexane	BJ 216-4	Lot no. B1153
1-Ethyl-3-nitro-1-nitroso-guanidine	Aldrich E4, 160-5	Lot no. 08930PK
Ethyl Ether	Fisher 138-4	Lot no. 956033-15

[2-^{14}C]Glycine, N-(2-ethoxy-2-oxo[1-^{14}C]ethyl)-N-methyl ethyl ester ([^{14}C]-6)

Methylamine hydrochloride (63 mg, 0.95 mmol) was dissolved in ethanol (3 mL) with sonication to effect dissolution. The [^{14}C]-**4** prepared above was dissolved in ethanol (0.5 mL) and added to the first solution. The [^{14}C]-**4** was rinsed in with additional ethanol (3 x 300 µL and 2 x 500 µL). A solution of triethylamine (294 mg, 2.91 mmol) in ethanol (9 mL) was added dropwise over 2 h. The resulting mixture was stirred for 64 h at room temperature. The ethanol was stripped and the residue dissolved in methylene chloride and washed with water. The water layer was extracted with methylene chloride three times. The methylene chloride solutions were combined, washed once with water, dried (Na_2SO_4), counted (46.6 mCi) and stripped. The residue of crude diester [^{14}C]-**6** was combined with additional [^{14}C]-**6** from an earlier preparation (CT-8136-155) and bulb to bulb distilled at 0.01 Torr. This distillation was conducted by holding the pot at room temperature and a receiver at liquid nitrogen temperature for 15 min. This removed traces of methylene chloride and any ethoxyethyl [2-^{14}C]acetate that may have formed. The remaining residue was then bulb to bulb distilled at 100 °C. The product was collected in a bulb cooled with evaporating methyl chloride while any remaining very volatile material would go on further to a receiver cooled with liquid nitrogen. A total of 288 mg of [^{14}C]-**6** was collected. A ^{1}H NMR spectrum of the distillate was consistent with the desired product.

Chemicals and Sources

Methylamine Hydrochloride	Aldrich 24,101-6	Lot no. 10924CG
Ethanol	Aaper	Lot no. 102464
Triethylamine	Aldrich 13,206-3	Lot no. 06110EW
Methylene Chloride	BJ 300-4	Lot no. B 1855
Sodium Sulfate	Baker 3898-D	Lot no. F29169

[1,1'-^{14}C]-2-Hydroxy-N-(2-hydroxyethyl)-N-methylethanamine ([^{14}C]-7)

A solution of lithium aluminum hydride (LAH) 240 mg (6.32 mmol) in tetrahydrofuran (THF) (7 mL, freshly distilled from benzophenone ketyl) was prepared. A solution of the diester [^{14}C]-**6** (288 mg, 1.41 mmol) in THF (1 mL) was added dropwise over 2 min to the solution of LAH. The diester was rinsed in with additional THF (4 x 0.5 mL). The resulting mixture was stirred overnight at room temperature. The excess LAH was destroyed by careful dropwise addition of saturated sodium sulfate solution. The THF was stripped from the reaction. Using water and 50% sodium hydroxide the reaction residue was transferred to a continuous extractor. A total of 19 mL of water and 1.5 mL of 50% sodium hydroxide solution was used for this transfer. The resulting solution was extracted with Et_2O for 42 h. The Et_2O solution was replaced with fresh Et_2O and extracted for 24 hrs. Three additional changes of Et_2O followed by 24 hrs of extraction gave a total of five extracts. Each of these were counted and gave respectively, 28.3, 12.9, 13.3, 6.13, and 0.78 mCi. Each of these extracts were analyzed by TLC (SiO_2-$CHCl_3$:CH_3OH:NH_4OH, 20:5:1) and it was found that the first extract was ~ 90% pure while the remaining extracts were >97% pure. The first extract was slowly evaporated under a stream of nitrogen in a 25-mL flask. The flask was placed on the vacuum manifold (0.01 Torr) at 0 °C to remove any remaining Et_2O. The residue weighed 70 mg and was used in the next reaction. Extracts 2-5 were combined and stored at -70 °C for possible use in the future.

Chemicals and Sources

Lithium Aluminum Hydride	Aldrich 19,987-7	Lot no. 01628TY
Sodium Hydroxide	EM-SX0590	Lot no. 30319048
Ethyl Ether	Fisher 138-4	Lot no. 9 56033-15
Sodium Sulfate	Baker 3898-D	Lot no. F29169

2,2'-Dichloro-N-methyldi([2-^{14}C]ethyl)amine Hydrochloride ([^{14}C]-8)

Diol [^{14}C]-**8** (extract 1, 28.3 mCi) prepared above was dissolved in benzene (0.5 mL) and added dropwise to a solution of thionyl chloride (215 mg, 1.8 mmol, 131 μL) in benzene (1 mL). The diol was rinsed in with additional benzene (3 x 0.5 mL). The resulting solution was warmed at 55-60 °C for 2 h and then allowed to cool and stir overnight at room temperature. The reaction mixture containing a small amount of white solid was stripped to remove benzene and excess thionyl chloride. The residue was dried at 0.01 Torr for 20 min. The dried residue was dissolved in ethanol (2 mL) and then stripped to dryness. The resulting product was dried at 0.01 Torr for 20 min. The dried product was dissolved in hot acetone (3 mL), treated with a small amount of activated charcoal and then filtered through a 0.45 μm Teflon filter into a tared 15 mL centrifuge tube. The solution was evaporated to dryness with a slow stream of nitrogen. The residue was dissolved in hot acetone (350 μL) and Et_2O (100 μL) was added. The resulting light yellow solution was allowed to cool to room temperature. After one hour no crystals had formed, so additional Et_2O (100 μL) was added. Again, after one hour no crystals had formed so a seed crystal of unlabeled **8** was added. Crystals started forming after ~ 1 min. The mixture was allowed to stand overnight. The crystals were centrifuged and the supernatant removed with a pipette. The crystals were rinsed with acetone-Et_2O (3.5:2, 2 x 300 μL) and then Et_2O (2 x 500 μL). The solid was dried at 0.01 Torr for one hour to give 71 mg of off-white crystals (CT-8136-179-1) and 22 mg of mother liquor residue. A specific activity determination on CT-8136-179-1 gave a value of 47 mCi/mmol. The mother liquor residue plus unlabeled **8** (6 mg) was recrystallized from hot acetone (300 μL) and a small amount of Et_2O. The crystals were centrifuged, rinsed with 60% acetone-Et_2O (2 x 300 μL) and dried at 0.01 Torr to give 13 mg of [^{14}C]-**8**, CT-8136-179-2. The mother liquor was discarded. CT-8136-179-2 and unlabeled **8** (44 mg) were dissolved in acetone, treated with activated charcoal, filtered through a 0.45 μm Teflon filter and evaporated to dryness under a stream of nitrogen. The residue was dissolved in hot acetone (450 μL) and Et_2O (50 μL) was added, the

resulting solution allowed to cool over 3 h. The crystals were centrifuged, the supernatant pipetted out and the solid rinsed with 66% acetone-Et_2O (3 x 200 µL). After drying for 3 h in vacuo (0.01 Torr), 47 mg of white crystals were obtained (CT-8136-179-3).

Unlabeled **8**, CT-8136-179-1 (71 mg) and CT-8136-179-3 (47 mg) were dissolved in hot acetone (650 µL) and Et_2O (100 µL) was added. A white solid immediately precipitated. Approximately 20% of the solvent was evaporated with a nitrogen stream, and additional acetone (200 µL) was added. The mixture was heated to effect dissolution and this solution allowed to stand overnight. The solid was centrifuged and the supernatant removed. The solid was rinsed with acetone (1 x 100 µL, 1 x 200 µL), 80% acetone-Et_2O (1 x 300 µL) and Et_2O (1 x 500 µL, 1 x 1000 µL). This solid was dried for 3 h in vacuo to obtain 70 mg of white crystals CT-8136-181-1. The mother liquor was evaporated to give 77 mg of white solid CT-8136-181-2. Unlabeled **8** (17 mg) and CT-8136-181-1 (70 mg) were dissolved in acetone (~ 800 µL). This solution was treated with activated charcoal, filtered through a 0.45 µm Teflon filter and evaporated to dryness with a stream of nitrogen. The residue was recrystallized from hot acetone (800 µL) to obtain 58 mg of shiny white crystals (CT-8136-181-3). This material was found to be 98% radiochemically pure by TLC on reverse phase RP-18 plates using 4% toluenesulphonic acid in acetone. This sample melted at 105-108 °C (sweating at 104 °C). A commercial sample of **8** had a mp of 104-108 °C (sweating at 98 °C). A specific activity determination gave a value of 88.5 µCi/mg. The ^1H NMR spectrum of CT-8136-181-3 was identical with that of a commercial sample of **8**. The mother liquor was evaporated to give 26 mg of white solid (CT-8136-181-4).

The mother liquor residue CT-8136-181-2 was recrystallized from hot acetone to give 55 mg of white crystals (CT-8136-181-5) and 28 mg of mother liquor (CT-8136-181-6). A specific activity determination gave a value of 111 µCi/mg for CT-8136-181-5. Unlabeled **8** (25 mg), CT-8136-181-5 (55 mg) and CT-8136-181-4 (26 mg) were recrystallized from hot acetone (600 µL) to give 77 mg of white crystals (CT-8136-181-7) and a mother liquor residue of 26 mg (CT-8136-181-8). The radiochemical purity of

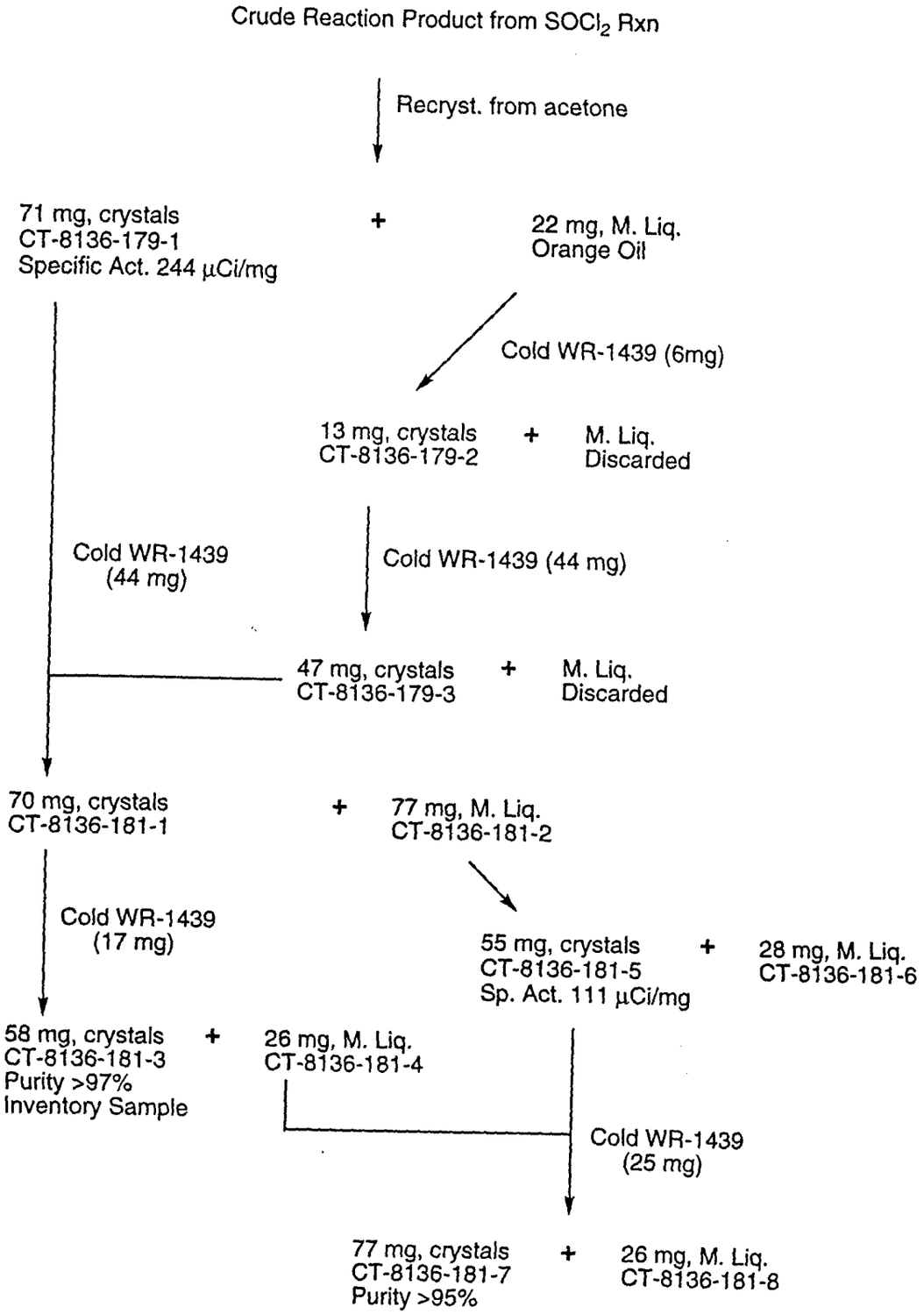
CT-8136-181-7 was determined to be 95% (RP-18: 4% toluenesulphonic acid in acetone).

Sample CT-8136-181-3 of [^{14}C]-**8** was entered into the inventory and is available for shipment. All other samples are being stored for further processing, if required.

Chemicals and Sources

Thionyl Chloride	Eastman 246	Lot no. 10924CG
Benzene	EM BX0212-6	Lot no. 34302
Ethanol	Aaper	Lot no. 102464
Acetone	Fisher A18-4	Lot no. 933703
Mechlorethamine Hydro-chloride (**8**) WR-1439	Aldrich 12,256-4	Lot no. 05006MF

Flow Sheet for Crystallization of [^{14}C]WR-1439



Synthesis Report

Walter Reed Institute of Research

Contract DAMD17-93-C-3001

1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N,N-di-n-butylamino)-[1-^{14}C]propyl]phenanthrene Hydrochloride

WR-171669

Lot No. CF-8144-182-3

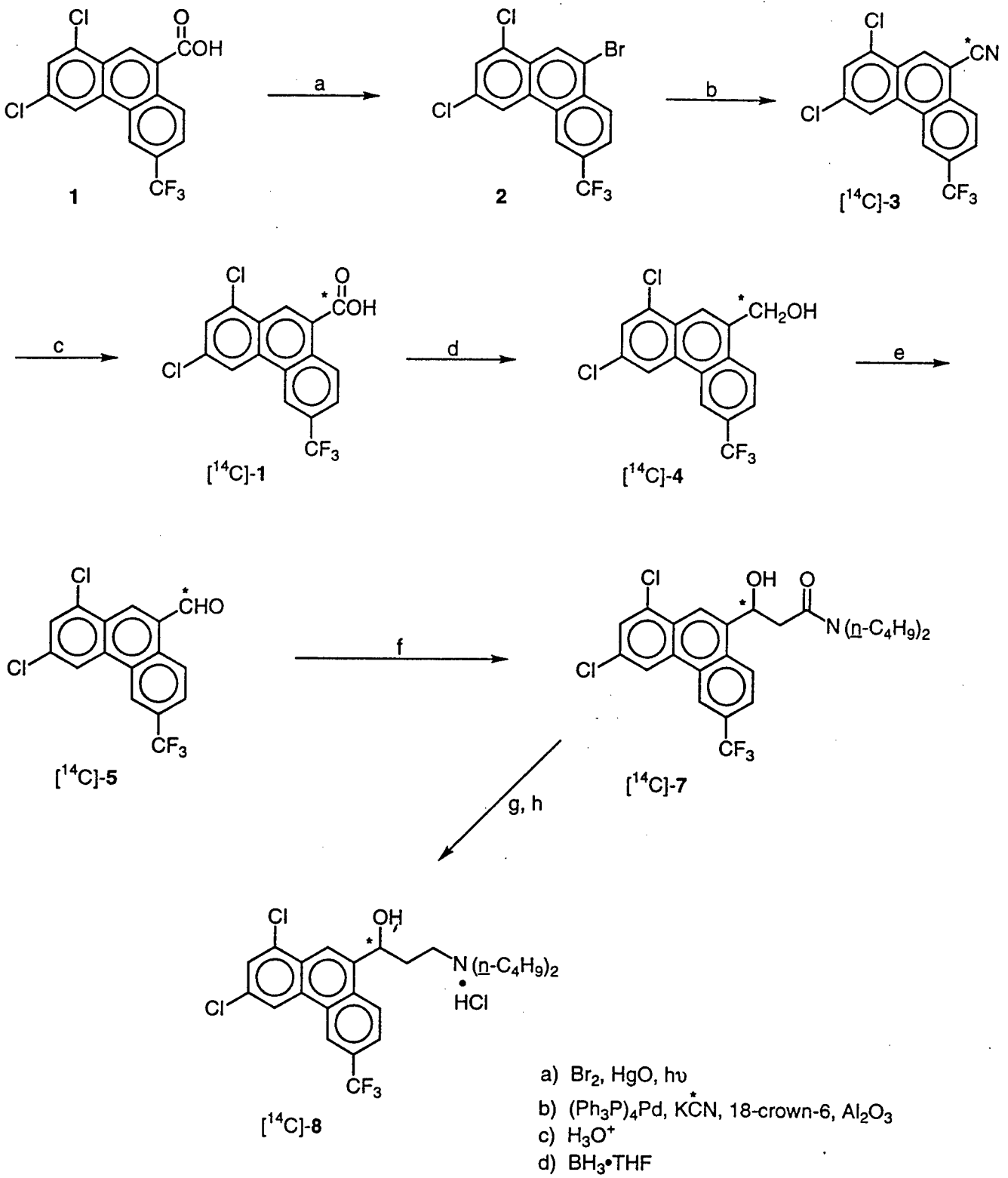
November 1995

C. D. Friedrich
J. A. Kepler

Research Triangle Institute
Research Triangle Park, NC 27709

Experimental

Proton NMR spectra were recorded on a Bruker AM250 spectrometer. Analytical TLC were performed using E. Merck silica-gel 60 F-254 plates for normal phase. Column chromatographies were performed with E. Merck silica gel 60 (230 - 400 mesh). Radioactive samples were counted on a Packard Tri-carb 4000 liquid scintillation counter. Developed TLC plates were scanned on a Berthold model LB 285 Linear Analyzer system. HPLC-RAM was done using a Waters Associates Model 6000A dual pump system with a Model U6K septumless injector and a IN/US System, Inc. Model 20725 β-RAM Flow-through Monitor. Solvents were removed from solutions on a rotary evaporator under water aspirator vacuum and ambient temperature unless otherwise noted.


1
a
2
b
[14C]-3
c
[14C]-1
d
[14C]-4
e
[14C]-5
f
[14C]-7
g, h
[14C]-8
a) Br2, HgO, hυ
b) (Ph3P)4Pd, K*CN, 18-crown-6, Al2O3
c) H3O+
d) BH3•THF
e) (NH4)2Ce(NO3)6, (CAN)
f) BrZnCH2CN(n-C4H9)2 (6)
g) BH3•THF
h) HCl

1,3-Dichloro-6-trifluoromethyl-9-bromophenanthrene (2)

1,3-Dichloro-6-trifluoromethyl-9-phenanthroic carboxylic acid (**1**, 10 g; 27.85 mmol) mercury(II) oxide (red) (15 g, 69.25 mmol), and 1,2-dichloroethane (250 mL) were added to a 1000-mL 3-neck RBF, (fitted with a reflux condenser, dropping funnel and stopper), and the mixture was stirred at 80 °C. The reaction mixture was irradiated with a UV lamp and bromine (2.5 mL, 48.52 mmol) diluted to 5 mL with dichloroethane, was added over 5 min, turning the solution orange. The lamp was kept on for 10 more minutes. Analysis by TLC indicated that no **1** remained and the lamp was turned off (15 min total). Charcoal was added, and the reaction mixture was filtered through a medium frit while still hot. The solvent was stripped. Heptane was added to the residue and the mixture was heated to reflux with a heat gun; sonicated, refluxed again, and allowed to cool to room temperature. After adding alumina (~ 50 g), the mixture was stirred and allowed to stand for ~ 5 min. Silica gel (~50 g) was added and the mixture allowed to stand for 10 min. These additions of Al_2O_3 and SiO_2 were made until the orange solution became virtually colorless. The solution was then filtered and stripped. The residue was dissolved in heptane and put through a flash column (30 mm ID, 15.2 cm silica, 5 cm alumina). The heptane was stripped to give a 40% yield of **2** (4.40 g, 12.26 mmol). This sample was recrystallized twice from heptane to yield 25% of **2** (2.75 g, 7.00 mmol) >99% purity by HPLC (t_R 7.2 min).[1] This material was used as is for the next step.

Chemicals and Sources

1,3-dichloro-6-trifluoro-methyl-9-phenanthroic carboxylic acid	BN# E029674 BN# E672269	WRAIR ship #793371AI WRAIR ship #793371AI
mercury(II) oxide (red)	Aldrich 21,335-7	Lot no. 08427 PY
1,2-dichloroethane	Aldrich 31,992-9	Lot no. 00527 BZ
bromine	Aldrich 27,757-6	Lot no. 08721 HW
heptane	Fisher H350-4	Lot no. 946729

alumina, basic	Aldrich 19,944-3	Lot no. 16504 CG
silica gel, 60	EM; 230-400 mesh; 9385-9	Lot no. 32027

1,3-Dichloro-6-trifluoromethyl-9-([^{14}C]cyano)phenanthrene ([^{14}C]-3)

Note: All glassware, pipettes, spatulas, and stirring bars were oven dried (122 °C) and cooled under N_2. All of the of the operations carried out with tetrakis(triphenylphosphine)palladium(0) (TTTP) was conducted in a glovebag under N_2.

18-Crown-6 (1.43 g, 2.17 mmol) and a stirring bar were placed in a 10-mL flask and dried on the vacuum manifold at 0.01-0.005 torr.[2,3] The 18-crown-6 was melted with the heat gun and stirred to remove residual water. Potassium [^{14}C]cyanide (248 mg, 3.70 mmol, 200 mCi, 54 mCi/mmol) was added to a 100-mL RBF containing a stirring bar and was dried on the vacuum manifold for ~ 10 min at 0.01-0.005 torr. **2** (1.68 g, 4.16 mmol) was added to the flask and the mixture was dried under vacuum with stirring. Alumina (209 mg, 2.05 mmol) was added to the flask and the mixture was again dried under vacuum for ~10 min. TTTP (876 mg, 0.758 mmol) was weighed in a vial in a glove bag and was added to the reaction flask. This operation was also carried out in the glovebag. The flask was attached to the vacuum manifold and dried at 0.01-0.005 torr for ~15 min. The flask was removed from the manifold, was fitted with a reflux condenser, and benzene (43 mL) was added. A solution of the dried 18-crown-6 in 1 mL of benzene was added to the flask. The reaction was refluxed at 95 °C with stirring for a total of 23 h when analysis by TLC indicated that the reaction was complete. The reaction was allowed to cool to room temperature and the benzene was stripped. Chloroform (~ 30 mL) was added to the crude product and the suspension was filtered through a cotton-sand plug to remove the Al_2O_3. The column was washed successively with methanol (~ 25 mL), tetrahydrofuran (~ 100 mL), and acetone (~ 100 mL). Each wash was collected separately, analyzed by TLC and HPLC[1] and counted. The THF and acetone washes were put aside and used in the second column. The remainder of the crude mixture of [^{14}C]-**3** was chromatographed on a clean-up

column (10 mm column, 18 cm silica, 30% CH_2Cl_2-heptane). Fractions (15-20 mL each) containing [^{14}C]-**3** were combined with the acetone and THF washes and rechromatographed (30 mm column, 13 cm SiO_2, 100% heptane to 60% CH_2Cl_2-heptane). Fractions containing pure [^{14}C]-**3** were combined to afford 1.13 g (3.32 mmol, 163 mCi, 77.8% chemical and 81.6% radiochemical yield) of product: HPLC[1] (t_R 3 sec) and TLC-RAM (1:1 hexane-CH_2Cl_2, R_f 0.35).

Chemicals and Sources

potassium [^{14}C]cyanide	Amersham Life Sciences	CFQ8866
alumina, basic	Aldrich 19,944-3	Lot no. 16504CG
tetrakis(triphenylphosphine)-palladium(0)	Aldrich 21,666-6	Lot no. 15429LG
18-crown-6	Aldrich 18,665-1	Lot no. 04114DP
benzene	EM; BV0212-6	Lot no. 34302
chloroform	Fisher C606-4	Lot no. 952046
heptane	Fisher H350-4	Lot no. 946729
methylene chloride	B & J 300-4	Lot no. BJ731
tetrahydrofuran	Fisher T425-4	Lot no. 942527-12
acetone	Fisher A18-20	Lot no. 952922
chloroform-d	Aldrich 22,578-9	Lot no. 03010CN

1,3-Dichloro-6-trifluoromethyl-9-phenanthroic [^{14}C]Carboxylic Acid ([^{14}C]-1)

A sample of [^{14}C]-**3** (1.13 g, 3.32 mmol, 163 mCi), glacial acetic acid (220 mL), water (22 mL), sulfuric acid (17.4 mL) and concentrated hydrochloric acid (4.4 mL) were combined in a 500-mL RBF and stirred at reflux (120 °C) for 145 h. The solution was allowed to cool to room temperature and then treated with 229 mL of H_2O and stirred at room temperature for 3 h. The reaction was cooled to 10 °C (ice-bath) and stirred for 1 h. The residue was removed by filtration and washed with ~ 460 mL of H_2O. After air drying, the residue was washed with THF (~ 60 mL). The THF was stripped and the

product dried on the manifold (0.01 torr). Recrystallization from toluene afforded 1.13 g (3.15 mmol, 163 mCi, 94.7% chemical, 99.6% radiochemical yield) of [^{14}C]-**1**: TLC-RAM (100:1 EtOAc-HOAc, R_f 0.48).

Chemicals and Sources

glacial acetic acid	Fisher A38^{C}-212	Lot no. 945572
sulfuric acid	Fisher A300^{C}-212	Lot no. FL-04-390
conc. hydrochloric acid	Fisher A144^{C}-212	Lot no. 943881
tetrahydrofuran	Fisher T425-4	Lot no. 942527-12
toluene	B&J 347-4	Lot no. BJ667
tetrahydrofuran-d_8	Aldrich 26,940-0	Lot no. 00525JV

1,3-Dichloro-6-trifluoromethyl-9-phenanthryl[^{14}C]carbinol ([^{14}C]-4)

A sample of [^{14}C]-**1** (1.13 g, 3.14 mmol, 163 mCi) and tetrahydrofuran (freshly distilled) (28.6 mL) in a 100-mL RBF was cooled to 0 °C by an ice-bath. Borane-tetrahydrofuran complex (10.8 mL) was added dropwise to the stirred mixture. The reaction was stirred for 30 min at 0 °C and then refluxed at 70 °C for 5.5 h. After cooling to room temperature, 9 mL of H_2O was added and stirring continued for ~ 15 min. The solvent was stripped to leave a creamy white solid. The residue was treated with 63 mL of H_2O and 1.6 mL of 50% NaOH solution, and the mixture was rapidly stirred for ~ 30 min. The mixture was filtered through a medium frit and the flask and frit were washed with a total of 400 mL of H_2O. After air drying, the residue was washed through the frit with THF (~ 50 mL). Removal of the solvent and crystallization of the residue from heptane, gave pure [^{14}C]-**4** in 64.9% chemical yield and 58% radiochemical yield (705 mg, 2.04 mmol, 95 mCi, 52 mCi/mmol): TLC-RAM (1:1 EtOAc-CH_2Cl_2, R_f 0.55). (The low yield was due to an overflow accident from the addition of H_2O to the reaction, resulting in the loss of about 45 mCi).

Chemicals and Sources

borane-tetrahydrofuran complex	Aldrich 17,619-2	Lot no. 00125AG
tetrahydrofuran	Freshly Distilled from benzo-phenone ketyl	----------
Sodium hydroxide-50%	Fisher SS254-4	Lot no. 920871-24
tetrahydrofuran (in workup)	Fisher T425-4	Lot no. 942527-12
heptane	Fisher H350-4	Lot no. 946729
tetrahydrofuran-d_8	Aldrich 30,887-0	Lot no. 00705EG

1,3-Dichloro-6-trifluoromethyl-9-phenanthryl[^{14}C]carboxaldehyde ([^{14}C]-5)

A solution of ceric ammonium nitrate (CAN) (1.16 g, 2.12 mmol) in H_2O (3 mL) was added dropwise, over 10 min, to a solution of [^{14}C]-**4** (320 mg, 0.928 mmol, 47.6 mCi) in glacial acetic acid (9.63 mL) at 95 °C. This solution was stirred for 7 h, cooled to room temperature and treated with 54 mL of H_2O. The precipitate formed was collected by filtration and washed with H_2O (~ 150 mL) and air-dried. The dried residue was rinsed through the filter with THF. The solvent was stripped and the residue was dried on the manifold (0.01 torr) at 60 °C. Crystallization from heptane afforded a 71% chemical yield (227 mg) of [^{14}C]-**5** . Analysis of this material by TLC-RAM indicated that further purification was needed. It was combined with a second crop of [^{14}C]-**5** and chromatographed (20 mm column, 18.5 cm silica, CH_2Cl_2). Fractions containing pure [^{14}C]-**5** were combined. Other fractions were combined to give material that was 91% radiochemically pure by TLC-RAM. This material was further purified by crystallization and combined with pure [^{14}C]-**5** from the chromatography to afford a 73% radiochemical yield (229 mg, 0.668 mmol, 34.8 mCi) of [^{14}C]-**5** that was 97% radiochemically pure by TLC-RAM (CH_2Cl_2).

Chemicals and Sources

cerium ammonium nitrate (CAN)	Janssen & Chimica, L1555-1	Lot no. 44131/3
glacial acetic acid	Fisher, A38^{c}-212	Lot no. 945572
tetrahydrofuran	Fisher T425-4	Lot no. 942527-12
heptane	Fisher H350-4	Lot no. 946474
methylene chloride	B & J, 300-4	Lot no. BK920

N,N-Di(n-butyl)-3-hydroxy-3-(1,3-dichloro-6-trifluoromethylphenanthren-9-yl)-[1-^{14}C]propionamide ([^{14}C]-7)

NOTE: All glassware was dried in the oven (122 °C) and cooled under N_2. All operations were carried out under a dry N_2 atmosphere.

Zinc (114 mg, 1.743 mmol) and benzene (30 mL) were placed in a 100-mL one-neck flask fitted with a Claisen adapter. Attached to the adapter were a dropping funnel and a Dean-Stark trap with condenser (setup A). Approximately 20 mL of benzene was distilled from the flask via the Dean-Stark trap. Heating was continued to maintain the mixture at reflux. A solution of N,N-di-(n-butyl)bromoacetamide[4,5] (245 mg, 0.984 mmol) in benzene (30 mL) was placed in a 100-mL two-necked flask fitted with a stopper and a Dean-Stark trap with condenser (setup B). Approximately 20 mL of benzene was distilled from the solution via the Dean-Stark trap. The solution was allowed to cool until refluxing ceased and the hot solution was transferred to the dropping funnel of setup A via a cannula by argon pressure. This solution was added dropwise over 30 min to the zinc suspension at reflux temperature. Refluxing was continued for 1 h after the addition was complete. A solution of [^{14}C]-**5** (229 mg, 0.668 mmol, 34.8 mCi) in 40 mL of benzene was placed in setup B and approximately 20 mL of benzene was distilled from the solution via the Dean-Stark trap. The dried solution was cooled until refluxing ceased and was transferred to the dropping funnel of setup A via a cannula by argon pressure. The solution was added dropwise over

30 min at reflux to the reaction mixture. Heating at reflux continued for 4.5 h after the addition was complete. The progress of the reaction was check periodically by TLC-RAM and was stopped when no more [^{14}C]-**5** could be detected. The reaction was cooled to room temperature and filtered hot through a celite pad to remove unreacted zinc. The celite was washed with benzene (25 mL) and CH_2Cl_2 (25 mL). The combined filtrate and washings was washed successively with 2 x 3 mL of sulfuric acid (10% solution), 4 x 3 mL of water, 2 x 3 mL of sodium chloride (saturated solution) and dried over sodium sulfate. The dried solution was filtered through a teflon acrodisc into a flask. The solvent was stripped to afford [^{14}C]-**7** that was 92% radiochemically pure by TLC-RAM (0.8% MeOH-CH_2Cl_2). Further purification was accomplished by flash column chromatography [30 mm I.D., 10.2 cm silica, CH_2Cl_2 (300 mL), 0.1% MeOH-CH_2Cl_2 (200 mL), 0.5% MeOH-CH_2Cl_2 (250 mL), and 0.8% MeOH-CH_2Cl_2 (250 mL)] while collecting 20 mL fractions. Fractions 26-33 containing pure [^{14}C]-**7** were combined and evaporated to afford 304 mg (0.591 mmol, 30.5 mCi; 88.6% chemical yield, 87.5% radiochemical yield) of material with specific activity of 51.5 mCi/mmol and 98% radiochemical purity by TLC-RAM (2% MeOH-CH_2Cl_2).

Chemicals and Sources

N,N-di-n-butyl-bromo-acetamide	RTI	Lot no. CF-8144-21
zinc, 30 mesh-granular	Fisher, Z-16-80072	Lot no. 745822
benzene	EM, BX0212-6	Lot no. 34302
methylene chloride	B & J, 300-6	Lot no. BK920
sulfuric acid	Fisher, A300^{C}-212	Lot no. FL-04-0390
sodium chloride	Fisher, S640-500	Lot no. 917093C
sodium sulfate	Baker, 3898-01	Lot no. J04157
methanol	B & J, 230-4	Lot no. BK513
chloroform-d	Aldrich, 22,578-9	Lot no. 03010CN

1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N,N-di-n-butylamino)[1-^{14}C]propyl]-phenanthrene Hydrochloride ([^{14}C]-8)

Borane-tetrahydrofuran (1.0 M, 1.9 mL, 1.9 mmol) was added dropwise to a solution of [^{14}C]-**7** (304 mg, 0.591 mmol, 30.5 mCi) in ether (6.9 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1.75 h, room temperature for 1 h and at reflux for 7 h. The reaction was allowed to cool to room temperature, quenched with 16.9 mL of H_2O, washed successively with 3 x 7 mL of 3 N hydrochloric acid, 3 x 7 mL of 10% sodium bicarbonate solution, and 2 x 7 mL of water. The organic layer was dried over sodium sulfate, filtered, and stripped to afford the free base of [^{14}C]-**8** as a cloudy oil. The oil was dissolved in a mixture of methanol (6.9 mL) and concentrated hydrochloric acid (2.4 mL) and refluxed for 7 h to decompose any borane complexes. Analysis by TLC-RAM indicated less than 2% extraneous material present. The solvent was stripped and the oily residue dried on the manifold (0.01 torr). The product was crystallized from ether with a few drops of methanol to aid dissolution to afford 208 mg, (0.388 mmol, 21.8 mCi, 56.2 mCi/mmol) of [^{14}C]-**8** (66% chemical yield, 71.6% radiochemical yield). Analysis by TLC-RAM (MeOH) showed the product to be 98% radiochemically pure. This material was diluted with nonlabeled halofantrine **8**[6] to afford 351 mg (0.654 mmol, 19.5 mCi) of [^{14}C]halofantrine ([^{14}C]-**8**) with specific activity of 29.8 mCi/mmol (55.5 μCi/mg) which was 98% and 97% radiochemically pure by HPLC-RAM[7] and TLC-RAM (100% MeOH), respectively (t_R 12 min 17 sec and R_f 0.57). The ^{1}H NMR spectrum of the product was identical to that of an authentic sample of halofantrine. This was stored in the freezer at -70 °C and given lot #CF-8144-182-3.

Chemicals and Sources

ether, anhydrous	Aldrich, 0402SKF	Lot no. 08187LG
borane-tetrahydrofuran complex	Aldrich, 17,619-2	Lot no. 00125AG
methanol, anhydrous	Aldrich, 32,241-5	Lot no. 03738DN
conc. hydrochloric acid	Fisher, A144^c-212	Lot no. 943881

sodium bicarbonate	EM, SX0320-1	Lot no. 9160
sodium sulfate	Baker, 3898-01	Lot no. J04157
methanol (used for specific activity)	B & J, 230-4	Lot no. BK513
methyl-d_3 alcohol-d	Aldrich, 30,881-1	Lot no. 18405EN

References

1. MetaChem Spherisorb 5 μ 005-2, 250 x 4.6 mm; Flow 2 mL/min, 96% CH_3CN-H_2O, 290 nm = UV.
2. Yamamura, K.; Murahashi, S.-I. *Tetrahedron Lett.* **1977**, *50*, 4429.
3. Dalton, R. J.; Regen, S. L. *J. Org. Chem.* **1979**, *44* (24), 4443.
4. Weaver, W. E .; Whaley, W. M. *JACS* **1947**, *69*, 515.
5. Ref., Roger D. Austin, lot #2850-191-IV.
6. Authentic halofantrine BN#BB43914 from WRAIR.
7. Phenomenex, CN column, 5 μ Spherisorb, 250 x 4.6 mm (#006-0107-EO); 20:50:30 CH_3OH-CH_3CN-0.01 M aqueous NH_4CO_2 (adjusted to pH 3 with formic acid); flow = 1 mL/min, UV = 290 nm.

Synthesis Report

Walter Reed Institute of Research

Contract DAMD17-93-C-3001

[16-^{14}C]Artelinic Acid

WR-255663

Lot No. CT-8440-43

March 1996

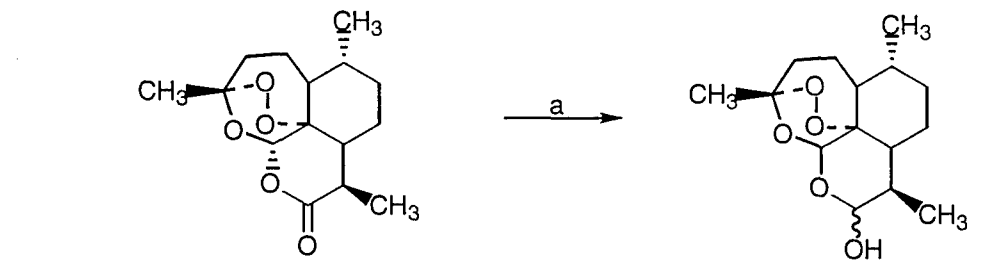
C. E. Twine
J. A. Kepler

Research Triangle Institute
Research Triangle Park, NC 27709

Experimental

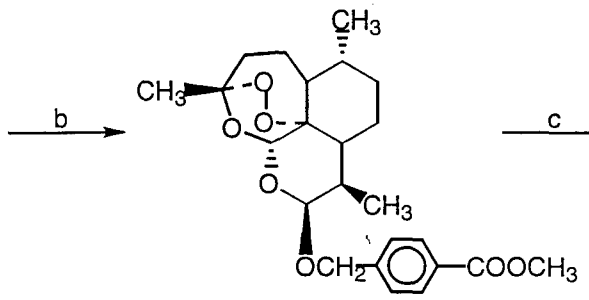
Analytical TLC were performed using E. Merck silica-gel F-254 plates. Column chromatographies were performed with Baker Silica Gel (40 μm Flash Chromatography Packing). Solvents were removed from solutions on a rotary evaporator under water aspirator vacuum and ambient temperature unless otherwise noted. Radioactive samples were counted on a Packard Tri-carb 4000 liquid scintillation spectrometer in Ultima Gold cocktail. Developed TLC plates were scanned on a Berthold model LB 283 Linear Analyzer system. HPLC was done using Waters Associates Model 6000A dual pump system with a Model U6K septumless injector and a Berthold Model LB503-HDS radioactivity monitor as the detector.

Synthetic Scheme

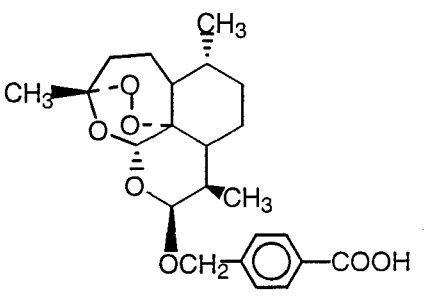


[^{14}C]-**1**

[^{14}C]-**2**



[^{14}C]-**3**



[^{14}C]-**4**

a) $NaBH_4$

b) $HOCH_2$–C$_6$H$_4$–$COOCH_3$

c) 5% KOH/CH_3OH

[16-^{14}C]Dihydroartemisinin ([^{14}C]-2)

A sample of [16-^{14}C]artemisinin (2.4 mCi, 102 μCi/mg, calcd. 23.5 mg) was dissolved in methanol (1.6 mL) and cooled to 0 °C with an ice bath. Sodium borohydride (30.3 mg, 0.80 mmol, 3.2 meq) was added in one portion and the resulting mixture stirred at 0 °C. After 2 h, TLC (SiO_2: 60% hexane-EtOAc) analysis indicated the reaction was complete. The reaction was worked up by adding enough acetic acid to be at least 150% times the number of meq of sodium borohydride. Thus, 1.4 mL (4.9 meq) of 20% acetic acid-methanol (3.49 M) was added to the reaction at 0 °C and this stirred for 15 min. The mixture was stripped to leave a white residue which was extracted with ethyl acetate (10 x 2 mL). The extracts were filtered through a cotton plug and then a 0.45 μm Teflon filter into a 25-mL volumetric flask. The solution was counted (2.4 mCi) and then the solvent was stripped and the residue stored overnight at -70 °C.

The crude [16-^{14}C]dihydroartemisinin was purified by flash chromatography on SiO_2 (10 mL) packed as a methylene chloride slurry in a 25-mL pipette. The following solvent mixtures were used while collecting the indicated fractions.

Fractions (5-6 mL)	**Solvent**
1,2	CH_2Cl_2
3-6	5% EtOAc-CH_2Cl_2
7-10	6% EtOAc-CH_2Cl_2
11,12	7% EtOAc-CH_2Cl_2
13,14	8% EtOAc-CH_2Cl_2
15-18	9% EtOAc-CH_2Cl_2
19-38	10% EtOAc-CH_2Cl_2

The fractions were analyzed by TLC (SiO_2; same system) and those fractions containing pure product (fractions 12-32) were combined, counted (1.91 mCi) and then stripped. The residue was stored overnight at -70 °C. The fractions containing impure product were combined and counted (67.5 μCi, 2.5% of the radioactivity). This was discarded.

Chemicals and Sources

[16-^{14}C]artemisinin	RTI	Lot no. GM-8160-109-7
methanol	Burdick & Jackson 230-4	Lot no. EL 430
sodium borohydride	Aldrich 19,807-2	Lot no. 02320 PX
acetic acid	Fisher A38-212	Lot no. 945572
silica gel	Baker 7024-01	Lot no. J07340
methylene chloride	Burdick & Jackson 300-4	Lot no. BJ731
ethyl acetate	Burdick & Jackson 100-4	Lot no. B1795

Methyl [16-^{14}C]artelinate ([^{14}C]-3)

To the 25-mL flask containing the [^{14}C]-**2** (1.9 mCi, calcd. 18.7 mg, 0.066 mmol) prepared above was added ethyl ether (3.2 mL) followed by methyl 4-(hydroxymethyl)-benzoate (39.5 mg, 0.24 mmol). To this turbid mixture was added boron trifluoride etherate (11.2 mg, 0.079 mmol, 1.2 meq, 9.8 μL). The mixture which had become clear was stirred at room temperature. After 64 h, TLC (SiO_2; 60% hexane-EtOAc) analysis indicated ~3.6% of [^{14}C]-**2** still present. Additional boron trifluoride etherate (3 μL, 3.5 mg, 0.024 mmol) was added and the solution stirred overnight. After a total of 88 h, TLC (same system) indicated the [^{14}C]-**2** was still present plus small amounts of a new impurity. The reaction was worked up by adding it to a separatory funnel containing saturated sodium bicarbonate solution (10 mL). The Et_2O layer was separated and washed with saturated sodium bicarbonate (2X) and brine. It was dried over Na_2SO_4, counted (1.86 mCi) and then stripped. The residue was flash chromatographed on SiO_2 (10 mL) packed as a 5% EtOAc-hexane slurry in a 25-mL pipette. A small amount of CH_2Cl_2 (< 1 mL) was needed to dissolve the material to allow it to be put on the column. The column was eluted with 5% EtOAc-hexane (~20 mL) and then the following solvents were used.

Fractions (5 mL)	Solvent
1,2	7% EtOAc-hexane
3-4	8% EtOAc-hexane
5-10	9% EtOAc-hexane
11-22	10% EtOAc-hexane
23-26	15% EtOAc-hexane

The fractions were analyzed by TLC (same system) and those containing pure [^{14}C]-**3** (Fractions11-19) were combined, counted (1.83 mCi) and then stripped. This material was used in the next reaction.

Chemicals and Sources

ethyl ether	Fisher E 138-4	Lot no. 956233-15
methyl 4-(hydroxymethyl)-benzoate	Aldrich 26,647-7	Lot no. 00416 PX
boron trifluoride etherate	Janssen 20,233,57	Lot no. 49878/1
sodium bicarbonate	Fisher S233-500	Lot no. 947459A
sodium chloride	Fisher S640-500	Lot no. 917093C
silica gel	Baker 7024-01	Lot no. J07340
ethyl acetate	Burdick & Jackson 100-4	Lot no. B1795
hexane	Burdick & Jackson 2164	Lot no. BE763

[16-^{14}C]Artelinic Acid ([^{14}C]-4)

The ester [^{14}C]-**3** (1.83 mCi, calcd. 27 mg, 0.064 mmol) was dissolved in methanol (500 μL). To this was added 5% aqueous potassium hydroxide solution (500 μL) and the resulting turbid mixture was stirred at room temperature under nitrogen. The turbidity cleared after ~30 min. After 4 h, TLC analysis (SiO_2: 60% EtOAc-hexane) indicated ~ 4% of [^{14}C]-**3** remained. After 22 h, TLC analysis (same system) indicated no change. Additional 5% potassium hydroxide solution (70 μL) was

added and mixture stirred at room temperature. After 4 h (26 h total), TLC analysis indicated no change. The reaction was stirred over the weekend. After 93 h, TLC (same system) indicated the reaction was complete. The reaction was worked up by adding acetic acid (84 mg, 1.4 mmol, 80 μL) and stirring at room temperature for 30 min. The mixture was stripped to leave a white residue. This was partitioned between Et_2O and H_2O. The Et_2O layer was separated and washed with H_2O (2X) and brine, dried over Na_2SO_4, counted (1.74 mCi) and then stripped. A sample was analyzed by HPLC[1] and found to contain ~ 5% of an impurity.

The crude product (~ 26 mg) was dissolved in CH_3OH (200 μL) and from this two injections of ~ 100 μL made on a preparative HPLC column.[2] The fractions were stripped to remove the CH_3OH and the resulting solutions extracted with Et_2O. The Et_2O extracts were washed with H_2O (2X), brine (1X) and dried over Na_2SO_4. The fractions were checked by analytical HPLC[1] and those containing pure [^{14}C]-**4** were combined and counted (1.58 mCi). This solution was dried under a stream of nitrogen, the residue dissolved in a small amount of Et_2O and filtered through a 0.45 μm Teflon filter into a 2-dram vial. The Et_2O was removed by evaporation under a stream of nitrogen. The residue was dissolved in a small amount of EtOAc (4 drops) and then hexane (500 μL) was added and the mixture allowed to crystallize. The supernatant was removed by pipette and evaporated. Both the crystals (15.5 mg) and mother liquor residue (9.2 mg) were dried on a vacuum manifold for 1 h. HPLC analysis[1] indicated both materials were >98% chemically pure. Unlabeled **4** (22.1 mg) which had also been purified by preparative HPLC[2] was dissolved in Et_2O and added to the mother liquor residue. The resulting solution was filtered through an 0.45 μm Teflon filter into the 2-dram vial containing the crystals of [^{14}C]-**4**. This solution was evaporated under a stream of nitrogen and the residue dissolved in a small amount of EtOAc. Hexane was added until the mixture started becoming turbid. This was allowed to crystallize for 3 h. The solvents were then carefully evaporated under a nitrogen stream and the resulting

material dried overnight on a vacuum manifold to yield 45 mg of white crystalline [^{14}C]-**4**. Its specific activity was determined to be 32.4 μCi/mg. Its chemical purity (UV) was 100% and its radiochemical purity was 99.7% by HPLC.[1] Its ^{1}H-NMR (500 mHz) was in agreement with a standard sample. This material was entered in the inventory as CT-8440-43.

Chemicals and Sources

methanol	Burdick & Jackson 230-4	Lot no. EL430
potassium hydroxide	Janssen 23.255.72	Lot no. 47003/1
acetic acid	Fisher A38c212	Lot no. 945572
ethyl ether	Fisher E 138-4	Lot no. 956233-15
sodium chloride	Fisher S640-500	Lot no. 917093C
sodium sulfate	Baker 3898-01	Lot no. F29169
ethyl acetate	Burdick & Jackson 100-4	Lot no. B1795
hexane	Burdick & Jackson 216-4	Lot no. BE763
ammonium acetate	Aldrich 23,807-4	Lot no. 10115KW
WR-255663	WRAIR	Lot no. BM04131

References

1. Waters μBondapak, C18, 10 μ, 3.9 x 300 mm, 65% CH_3OH–0.1 M $NH_4OAc(H_2O)$, 1.5 mL/min UV-235 nm.
2. Waters prep RCM μBondapak, C18, 10 μ, 25 x 200 mm, 65% CH_3OH–0.1 M $NH_4OAc(H_2O)$, 9.9 mL/min, UV-235 nm.

Synthesis Report

Walter Reed Institute of Research

Contract DAMD17-93-C-3001

1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N-*n*-butylamino)-[1-^{14}C]propyl]phenanthrene Hydrochloride

WR-178460

Lot No. CF-8448-129-4

July 1996

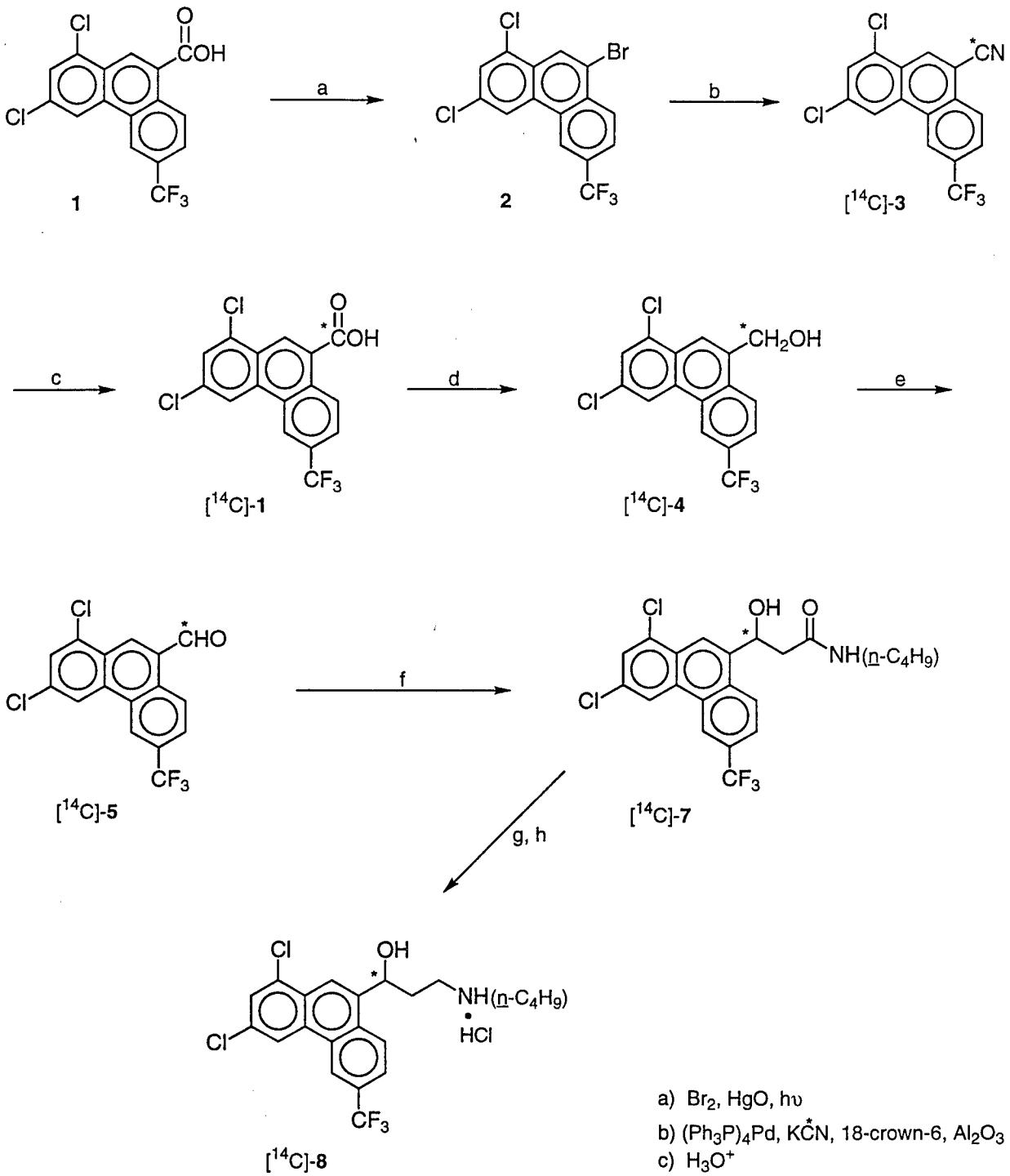
C. D. Friedrich
J. A. Kepler

Research Triangle Institute
Research Triangle Park, NC 27709

Experimental

Proton NMR spectra were recorded on a Bruker AM250 spectrometer. Analytical TLC were performed using E. Merck silica-gel 60 F-254 plates for normal phase. Column chromatographies were performed with E. Merck silica gel 60 (230-400 mesh). Radioactive samples were counted on a Packard Tri-carb 4000 liquid scintillation counter. Developed TLC plates were scanned on a Berthold model LB 285 Linear Analyzer system. HPLC-RAM was done using a Waters Associates Model 6000A dual pump system with a Model U6K septumless injector and a IN/US System, Inc. Model 20725 β-RAM Flow-through Monitor. Solvents were removed from solutions on a rotary evaporator under water aspirator vacuum and ambient temperature unless otherwise noted.

Synthetic Scheme



a) Br_2, HgO, hυ

b) $(Ph_3P)_4Pd$, K$\overset{*}{C}$N, 18-crown-6, Al_2O_3

c) H_3O^+

d) BH_3•THF

e) $(NH_4)_2Ce(NO_3)_6$, (CAN)

f) n-BuLi, $CH_3\overset{O}{\overset{\|}{C}}NH(n\text{-}C_4H_9)$ (**6**)

g) BH_3•THF

h) HCl

1,3-Dichloro-6-trifluoromethyl-9-bromophenanthrene (2)

1,3-Dichloro-6-trifluoromethyl-9-phenanthroic carboxylic acid (**1**, 10 g; 27.85 mmol) mercury(II) oxide (red) (15 g, 69.25 mmol), and 1,2-dichloroethane (250 mL) were added to a 1000-mL 3-neck RBF, (fitted with a reflux condenser, dropping funnel and stopper), and the mixture was stirred at 80 °C. The reaction mixture was irradiated with a UV lamp and bromine (2.5 mL, 48.52 mmol) diluted to 5 mL with dichloroethane, was added over 5 min, turning the solution orange. The lamp was kept on for 10 min more. Analysis by TLC indicated that no **1** remained and the lamp was turned off (15 min total). Charcoal was added, and the reaction mixture was filtered through a medium frit while still hot. The solvent was stripped. Heptane was added to the residue and the mixture was heated to reflux with a heat gun; sonicated, refluxed again, and allowed to cool to room temperature. After adding alumina (~ 50 g), the mixture was stirred and allowed to stand for ~ 5 min. Silica gel (~50 g) was added and the mixture allowed to stand for 10 min. These additions of Al_2O_3 and SiO_2 were made until the orange solution became virtually colorless. The solution was then filtered and stripped. The residue was dissolved in heptane and put through a flash column (30 mm ID, 15.2 cm silica, 5 cm alumina). The heptane was stripped to give a 40% yield of **2** (4.40 g, 12.26 mmol). This sample was recrystallized twice from heptane to yield 25% of **2** (2.75 g, 7.00 mmol) >99% purity by HPLC (t_R 7.2 min).[1] This material was used as is for the next step.

Chemicals and Sources

1,3-dichloro-6-trifluoro-methyl-9-phenanthroic carboxylic acid	BN# E029674 BN# E672269	WRAIR ship #793371Al WRAIR ship #793371Al
mercury(II) oxide (red)	Aldrich 21,335-7	Lot no. 08427 PY
1,2-dichloroethane	Aldrich 31,992-9	Lot no. 00527 BZ
bromine	Aldrich 27,757-6	Lot no. 08721 HW
heptane	Fisher H350-4	Lot no. 946729
alumina, basic	Aldrich 19,944-3	Lot no. 16504 CG
silica gel, 60	EM; 230-400 mesh; 9385-9	Lot no. 32027

1,3-Dichloro-6-trifluoromethyl-9-([^{14}C]cyano)phenanthrene ([^{14}C]-3)

Note: All glassware, pipettes, spatulas, and stirring bars were oven dried (122 °C) and cooled under N_2. All of the of the operations carried out with tetrakis(triphenylphosphine)palladium(0) (TTTP) was conducted in a glovebag under N_2.

18-Crown-6 (1.43 g, 2.17 mmol) and a stirring bar were placed in a 10-mL flask and dried on the vacuum manifold at 0.01-0.005 torr.[2,3] The 18-crown-6 was melted with the heat gun and stirred to remove residual water. Potassium [^{14}C]cyanide (248 mg, 3.70 mmol, 200 mCi, 54 mCi/mmol) was added to a 100-mL RBF containing a stirring bar and was dried on the vacuum manifold for ~10 min at 0.01-0.005 torr. **2** (1.68 g, 4.16 mmol) was added to the flask and the mixture was dried under vacuum with stirring. Alumina (209 mg, 2.05 mmol) was added to the flask and the mixture was again dried under vacuum for ~10 min. TTTP (876 mg, 0.758 mmol) was weighed in a vial in a glove bag and was added to the reaction flask. This operation was also carried out in the glovebag. The flask was attached to the vacuum manifold and dried at 0.01-0.005 torr for ~15 min. The flask was removed from the manifold, was fitted with a reflux condenser, and benzene (43 mL) was added. A solution of the dried 18-crown-6 in 1 mL of benzene was added to the flask. The reaction was refluxed at 95 °C with stirring for a total of 23 h when analysis by TLC indicated that the reaction was complete. The reaction was allowed to cool to room temperature and the benzene was stripped. Chloroform (~30 mL) was added to the crude product and the suspension was filtered through a cotton-sand plug to remove the Al_2O_3. The column was washed successively with methanol (~25 mL), tetrahydrofuran (~100 mL), and acetone (~100 mL). Each wash was collected separately, analyzed by TLC and HPLC[1] and counted. The THF and acetone washes were put aside and used in the second column. The remainder of the crude mixture of [^{14}C]-**3** was chromatographed on a clean-up column (10 mm column, 18 cm silica, 30% CH_2Cl_2-heptane). Fractions (15-20 mL each) containing [^{14}C]-**3** were combined with the acetone and THF washes and rechromatographed (30 mm column, 13 cm SiO_2, 100% heptane to 60% CH_2Cl_2-

heptane). Fractions containing pure [^{14}C]-**3** were combined to afford 1.13 g (3.32 mmol, 163 mCi, 77.8% chemical and 81.6% radiochemical yield) of product: HPLC[1] (t_R 3 sec) and TLC-RAM (1:1 hexane-CH_2Cl_2, R_f 0.35).

Chemicals and Sources

potassium [^{14}C]cyanide	Amersham Life Sciences	CFQ8866
alumina, basic	Aldrich 19,944-3	Lot no. 16504CG
tetrakis(triphenylphosphine)-palladium(0)	Aldrich 21,666-6	Lot no. 15429LG
18-crown-6	Aldrich 18,665-1	Lot no. 04114DP
benzene	EM; BV0212-6	Lot no. 34302
chloroform	Fisher C606-4	Lot no. 952046
heptane	Fisher H350-4	Lot no. 946729
methylene chloride	B & J 300-4	Lot no. BJ731
tetrahydrofuran	Fisher T425-4	Lot no. 942527-12
acetone	Fisher A18-20	Lot no. 952922
chloroform-d	Aldrich 22,578-9	Lot no. 03010CN

1,3-Dichloro-6-trifluoromethyl-9-phenanthroic [^{14}C]Carboxylic Acid ([^{14}C]-1)

A sample of [^{14}C]-**3** (1.13 g, 3.32 mmol, 163 mCi), glacial acetic acid (220 mL), water (22 mL), sulfuric acid (17.4 mL) and concentrated hydrochloric acid (4.4 mL) were combined in a 500-mL RBF and stirred at reflux (120 °C) for 145 h. The solution was allowed to cool to room temperature and then treated with 229 mL of H_2O and stirred at room temperature for 3 h. The reaction was cooled to 10 °C (ice-bath) and stirred for 1 h. The residue was removed by filtration and washed with ~ 460 mL of H_2O. After air drying, the residue was washed with THF (~ 60 mL). The THF was stripped and the product dried on the manifold (0.01 torr). Recrystallization from toluene afforded 1.13 g (3.15 mmol, 163 mCi, 94.7% chemical, 99.6% radiochemical yield) of [^{14}C]-**1**: TLC-RAM (100:1 EtOAc-HOAc, R_f 0.48).

Chemicals and Sources

glacial acetic acid	Fisher A38^{C}-212	Lot no. 945572
sulfuric acid	Fisher A300^{C}-212	Lot no. FL-04-390
conc. hydrochloric acid	Fisher A144^{C}-212	Lot no. 943881
tetrahydrofuran	Fisher T425-4	Lot no. 942527-12
toluene	B&J 347-4	Lot no. BJ667
tetrahydrofuran-d_8	Aldrich 26,940-0	Lot no. 00525JV

1,3-Dichloro-6-trifluoromethyl-9-phenanthryl[^{14}C]carbinol ([^{14}C]-4)

A sample of [^{14}C]-**1** (1.13 g, 3.14 mmol, 163 mCi) and tetrahydrofuran (freshly distilled) (28.6 mL) in a 100-mL RBF was cooled to 0 °C by an ice-bath. Borane-tetrahydrofuran complex (10.8 mL) was added dropwise to the stirred mixture. The reaction was stirred for 30 min at 0 °C and then refluxed at 70 °C for 5.5 h. After cooling to room temperature, 9 mL of H_2O was added and stirring continued for ~ 15 min. The solvent was stripped to leave a creamy white solid. The residue was treated with 63 mL of H_2O and 1.6 mL of 50% NaOH solution, and the mixture was rapidly stirred for ~30 min. The mixture was filtered through a medium frit and the flask and frit were washed with a total of 400 mL of H_2O. After air drying, the residue was washed through the frit with THF (~ 50 mL). Removal of the solvent and crystallization of the residue from heptane, gave pure [^{14}C]-**4** in 64.9% chemical yield and 58% radiochemical yield (705 mg, 2.04 mmol, 95 mCi, 52 mCi/mmol): TLC-RAM (1:1 EtOAc-CH_2Cl_2, R_f 0.55). (The low yield was due to an overflow accident from the addition of H_2O to the reaction, resulting in the loss of about 45 mCi).

Chemicals and Sources

borane-tetrahydrofuran complex	Aldrich 17,619-2	Lot no. 00125AG
tetrahydrofuran	Freshly Distilled from benzophenone ketyl	----------
Sodium hydroxide-50%	Fisher SS254-4	Lot no. 920871-24
tetrahydrofuran (in workup)	Fisher T425-4	Lot no. 942527-12

heptane	Fisher H350-4	Lot no. 946729
tetrahydrofuran-d_8	Aldrich 30,887-0	Lot no. 00705EG

1,3-Dichloro-6-trifluoromethyl-9-phenanthryl[^{14}C]carboxaldehyde ([^{14}C]-5)

A solution of ceric ammonium nitrate (CAN) (1.25 g, 2.28 mmol) in water (3.3 mL) was added dropwise to a stirred solution of [^{14}C]-**4**† (345 mg, 1.00 mmol, 46 mCi) in 10.4 mL of glacial acetic acid at 95 °C over a 15 min period. The solution was stirred until analysis by TLC-RAM (CH_2Cl_2) indicated the reaction was complete (~45 h‡). The reaction was cooled to ambient temperature and diluted with 60 mL of water. The precipitate was collected by filtration and washed with water (~ 300 mL) and air-dried. The dried residue was rinsed through the filter with THF. The solvent was removed from the solution and the residue dried on the vacuum line at 0.005 torr. The product was purified by flash column chromatography [20 mm diameter column, 150 mm silica gel, chloroform (pentane stabilized)] to afford 203 mg (32 mCi) of [^{14}C]-**5**.

Chemicals and Sources

cerium ammonium nitrate (CAN)	Janssen & Chimica, L1555-1	Lot no. 44131/3
glacial acetic acid	Fisher, A38^{C}-212	Lot no. 945572
tetrahydrofuran	freshly distilled from benzo-phenone ketyl	
chloroform (pentane)	Fisher C607-4	Lot no. 961410
heptane	Fisher H350-4	Lot no. 946474
silica gel	Aldrich 19,944-3	Lot no. 16504CG

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† This sample of [^{14}C]-**4** (lot no. CF-8144-159-3) had been stored for eight months at -70 °C. Anaylsis by TLC-RAM (1:1 EtOAc-CH_2Cl_2) indicated that it was 94% radio chemically pure.

‡ The time for this oxidation to go to completion has varied from 7 to 45 h in pilot runs.

N-(*n*-butyl)-3-hydroxy-3-(1,3-dichloro-6-trifluoromethylphenanthrene-9-yl)-[1-^{14}C]propionamide ([^{14}C]-7)

NOTE: All glassware was dried and cooled under an argon atmosphere.

N-Butylacetamide (**6**, 205 mg, 1.78 mmol) and a stirbar were placed in a two-neck flask fitted with a graduated dropping funnel and septum. Freshly distilled THF (8.1 mL) was transferred to the dropping funnel via a cannula, and then was added to the reaction flask. 1,10-Phenanthroline was added as an indicator. The mixture was cooled in a -5 °C cooling bath and *n*-butyllithium (1.6 M, 2.22 mL, 3.55 mmol) was added through the septum via a gastight syringe. The solution was stirred for 3 h. A solution of aldehyde [^{14}C]-**5** (200 mg, 0.58 mmol, 32 mCi) in 4.1 mL of THF was slowly added to the reaction mixture via a cannula. The mixture was stirred at -5 °C for 1.5 h and then at ambient temperature for 2 h. Aqueous ammonium acetate (13 mL of a 15% solution) was added and stirring continued for ~10 min. The solution was diluted with 30 mL of water before extraction with 70 mL of ethyl acetate. The ethyl acetate solution was back-washed with 3 x 70 mL of water. The solvent was removed after drying over sodium sulfate. The residue was purified by flash chromatography (20 mm diameter column, 205 mm silica gel) eluted as follows: 300 mL, $CHCl_3$; 200 ml, 10% $CHCl_3$-EtOAc; 200 mL, 20% $CHCl_3$-EtOAc; 300 mL, 30% $CHCl_3$-EtOAc; 200 mL, 50% $CHCl_3$-EtOAc. A 61.5% radiochemical yield (19.8 mCi, 157 mg, 0.34 mmol) of [^{14}C]-**7** was realized. Analysis by TLC-RAM (EtOAc) showed the sample was 98% radiochemically pure.

Chemicals and Sources

tetrahydrofuran	freshly distilled from benzophenone ketyl	
ethyl acetate	B & J, 100-4	Lot no. BJ081
chloroform (pentane)	Fisher C607-4	Lot no. 961410
acetone	Fisher A18-4	Lot no. 952917
N-butylacetamide	Acros, 403423-1000	Lot no. A008350601

sodium sulfate	Baker, 3898-01	Lot no. J04157
ammonium acetate	Aldrich, 23,807-4	Lot no. 10115KW
silica gel	Aldrich 19,944-3	Lot no. 16504CG

1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N-*n*-butylamino)[1-^{14}C]propyl]-phenanthrene Hydrochloride ([^{14}C]WR-178460, [^{14}C]-8)

Borane-THF complex (1 M, 1.56 mL, 1.56 mmol) was placed in a 25-mL flask fitted with a dropping funnel and was cooled in a -5 °C bath. A solution of amide [^{14}C]-**7** (157 mg, 0.343 mmol, 19.8 mCi) in 9.5 mL of THF was cannulated to the dropping funnel and then added to the cooled solution over 30 min. The solution was allowed to warm to ambient temperature and was then refluxed for 2 h. After cooling to ambient temperature, 1.6 mL of a 1:1 THF-H_2O solution was added. After stirring 10 min, 2.0 mL of water and 0.55 mL of concentrated hydrochloric acid were added to the mixture. This mixture was stirred at reflux temperature for ~1 h. The THF was distilled from the solution by using a short-path still and a bath temperature of 85 °C. The pot residue was carefully dried (0.005 torr) on the vacuum manifold. Analysis of the residue by TLC-RAM (40:5:0.8, $CHCl_3$-EtOH-NH_4OH) indicated that 20% of the starting material remained. The residue was extracted with ethyl acetate to remove unreacted amide [^{14}C]-**7**. The recovered [^{14}C]-**7** was combined with 80 mg of nonlabeled **7** (sample no. 8448-67-10) to afford 145 mg (0.317 mmol) of [^{14}C]-**7** of reduced specific activity. This sample was reduced as described above and the product combined in methanol with the [^{14}C]-**8** obtained from the original reduction to give 16 mCi (81% radiochemical yield) of product. Removal of the methanol afforded 211 mg (0.439 mmol) of [^{14}C]-**8** with specific activity of 75.8 μCi/mg (36.4 mCi/mmol). The radiochemical purity was 98% by HPLC-RAM [(Phenomenex Spherisorb CN, 5 μ, 4.6 x 250 mm; 20:50:30, CH_3OH-CH_3CN-0.01 M HCO_2NH_4 (pH adjusted to 3 with formic acid), 1 mL/min, 290 nm, t_R 5 min] and 97% by TLC-RAM (40:5:0.8, $CHCl_3$:EtOH:NH_4OH, R_f 0.5). The sample was given lot no. CF-8448-129-4.

Chemicals and Sources

tetrahydrofuran	freshly distilled from benzophenone ketyl	
borane-tetrahydrofuran complex	Aldrich, 17,619-2	Lot no. 00125AG, Lot no. 025524EX
methanol	B & J, 230-4	Lot no. BJ963
conc. hydrochloric acid	Fisher, A144^{c}-212	Lot no. 943881
ethyl acetate	B & J, 100-4	Lot no. BJ081

References

1. MetaChem Spherisorb 5 μ 005-2, 250 x 4.6 mm; Flow 2 mL/min, 96% CH_3CN-H_2O, 290 nm = UV.
2. Yamamura, K.; Murahashi, S.-I. *Tetrahedron Lett.* **1977**, *50*, 4429.
3. Dalton, R. J.; Regen, S. L. *J. Org. Chem.* **1979**, *44* (24), 4443.

Synthesis Report

Walter Reed Institute of Research

Contract DAMD17-93-C-3001

[16-^{14}C]Artemether

WR-254986

Lot No. CT-8440-77-1

May 1996

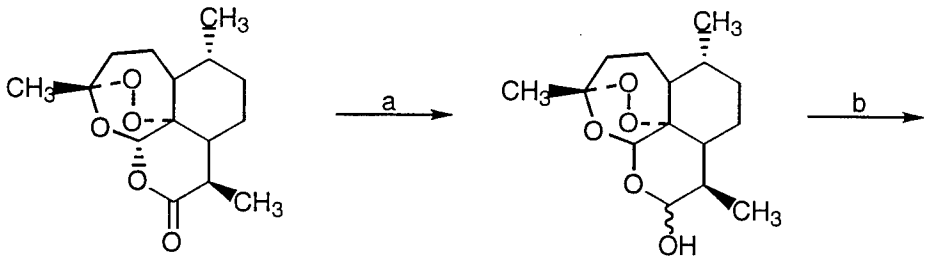
C. E. Twine
J. A. Kepler

Research Triangle Institute
Research Triangle Park, NC 27709

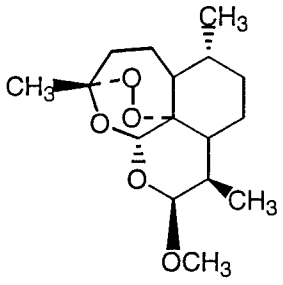
Experimental

Analytical TLC were performed using E. Merck silica-gel F-254 plates. Column chromatographies were performed with Baker Silica Gel (40 μm Flash Chromatography Packing). Solvents were removed from solutions on a rotary evaporator under water aspirator vacuum and ambient temperature unless otherwise noted. Radioactive samples were counted on a Packard Tri-carb 4000 liquid scintillation spectrometer in Ultima Gold cocktail. Developed TLC plates were scanned on a Berthold model LB 283 Linear Analyzer system. HPLC was done using Waters Associates Model 6000A dual pump system with a Model U6K septumless injector and a Berthold Model LB503-HDS radioactivity monitor as the detector.

Synthetic Scheme



[^{14}C]-**1** [^{14}C]-**2**



[^{14}C]-**3**

a) $NaBH_4$

b) CH_3OH, $BF_3 \cdot Et_2O$

[16-^{14}C]Dihydroartemisinin ([^{14}C]-2)

A sample of [16-^{14}C]artemisinin (1.0 mCi, 102 μCi/mg, calcd. 9.8 mg) was dissolved in methanol (1.2 mL) and cooled to 0 °C with an ice bath. Sodium borohydride (21 mg, 0.56 mmol, 2.2 meq) was added in one portion and the resulting mixture stirred at 0 °C for 2 h under nitrogen. TLC analysis (SiO_2: 60% hexane-EtOAc) indicated the reaction was complete. The reaction was stopped by adding enough acetic acid to be at least 150% times the number of meq of sodium borohydride used in the reaction. Thus, 0.95 mL (3.3 meq) of 20% HOAc-CH_3OH (3.49 M) was added at 0 °C and the resulting mixture stirred for 15 min. The mixture was stripped to leave a white residue. This was extracted with EtOAc (8 x 2 mL) and these extracts filtered through a cotton plug into a flask. The solution was stripped and the white residue was stored overnight at -70 °C.

The residue was flash chromatographed on SiO_2 (7 mL) packed as a methylene chloride slurry in a 25-mL pipette. The sample was put on the column in CH_2Cl_2. The following solvent mixtures were used to elute the column.

Fractions (5-6 mL)	**Solvent**
1,2	CH_2Cl_2
3,4	4% EtOAc-CH_2Cl_2
5,6	5% EtOAc-CH_2Cl_2
7-10	6% EtOAc-CH_2Cl_2
11,12	7% EtOAc-CH_2Cl_2
13-16	8% EtOAc-CH_2Cl_2
17,18	9% EtOAc-CH_2Cl_2
19-32	10% EtOAc-CH_2Cl_2

The fractions were analyzed by TLC (same system). Fractions 14-27 were found to contain pure [^{14}C]-**2** and were combined and counted (835 μCi). This was stored overnight at -70 °C. A small amount of [^{14}C]-**1** was found in fractions **4** and **5**.

Chemicals and Sources

[16-^{14}C]artemisinin	RTI	Lot no. GM-8160-109-7
methanol	Burdick & Jackson 230-4	Lot no. EL 430
sodium borohydride	Aldrich 19,807-2	Lot no. 02320 PX
acetic acid	Fisher A38c212	Lot no. 945572
ethyl acetate	Burdick & Jackson 100-4	Lot no. B1795
silica gel	Baker 7024-01	Lot no. J07340
methylene chloride	Burdick & Jackson 300-4	Lot no. BJ731

[16-^{14}C]Artemether ([^{14}C]-3)

The solution of [^{14}C]-**2** prepared above was stripped in a 25-mL pear shaped flask. The residue was dissolved in benzene (1 mL) and to this was added methanol (9.3 mg, 0.29 mmol, 11.7 μL) followed by boron trifluoride etherate (4.6 mg, 0.33 mmol, 4 μL). The mixture was turbid but became clear in ~5 min. The solution was stirred overnight under nitrogen at room temperature. After 17 h, TLC analysis (SiO_2: 60% hexane-EtOAc) indicated ~2% of [^{14}C]-**2** still remained. After 22.5 h, the reaction was worked up by adding saturated sodium acetate solution (3 mL) and stirring for 15 min. This mixture was then extracted with EtOAc (3X) and the combined extracts washed with H_2O (2X). The resulting solution was dried over Na_2SO_4 for 16 h. The solution was filtered into a volumetric flask, counted (830 μCi) and then evaporated under a stream of nitrogen. The residue was dissolved in toluene (~10 mL) and stored at -70°C. The toluene was stripped and the residue flash chromatographed on SiO_2 (10 mL) packed as a 3% EtOAc-petroleum ether slurry in a 25-mL pipette. The following solvent mixtures were used to elute the column.

Fractions	Fraction Size (mL)	Solvent
1-3	8	3% EtOAc-pet. ether
4-11	6	4% EtOAc-pet. ether
12-15	5	5% EtOAc-pet. ether
16-20	5	6% EtOAc-pet. ether
21-	5	8% EtOAc-pet. ether

The fractions were analyzed by TLC (same system) and the following was found.

Fraction 6	Impure [^{14}C]-**3**, β isomer
Fractions 7-11	Pure [^{14}C]-**3**, β isomer
Fractions 12-14	Impure [^{14}C]-**3**, β isomer
Fractions 15-19	Mixtures of [^{14}C]-**3**, α and β isomers
Fractions 20-28	Pure [^{14}C]-**3**, α isomer

Fractions 7-11 were combined, counted (451 μCi) and evaporated under a stream of nitrogen and redissolved in toluene for storage. Its radiochemical purity was determined to be 98% by HPLC (Beckman Ultrasphere ODS, 5 μ, 4.6 x 250 mm, 60% CH_3CN-H_2O, 1.2 mL/min, t_R 17 min 0 sec. Its specific activity is 96 μCi/mg. This material was entered into the inventory as CT-8440-77-1.

Chemicals and Sources

benzene	EM BX0212-6	Lot no. 34302
methanol	Aldrich 32,241-5	Lot no. 02211BY
boron trifluoride etherate	Janssen 20,233,57	Lot no. 49878/1
sodium acetate	EM SX0255-1	Lot no. 33356407
ethyl acetate	Burdick & Jackson 100-4	Lot no. B1795
sodium sulfate	Baker 3898-01	Lot no. F29169
toluene	Burdick & Jackson 347-4	Lot no. B1143
silica gel	Baker 7024-01	Lot no. J07340
petroleum ether	Burdick & Jackson 317-4	Lot no. BK269